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The problem of childhood hypoglycemia

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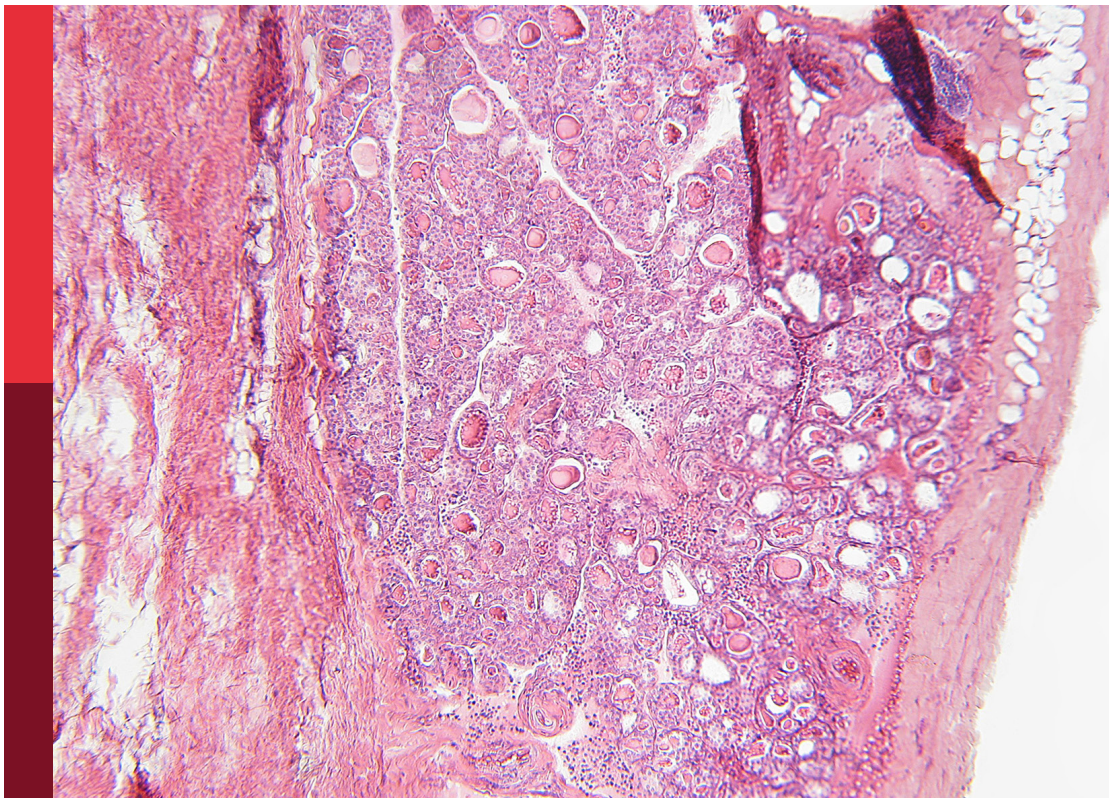

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The problem of childhood hypoglycemia

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Editorial: The problem of childhood hypoglycaemia

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KEYWORDS

hypoglycaemia, children, research, patient, patient – centered care, outcome, congenital hyperinsulinism

Editorial on the Research Topic
The problem of childhood hypoglycaemia

Hypoglycaemia is a common problem with significant adverse influence on neurodevelopment in childhood and lasting impact in adult life with consequent neurodisability. Hypoglycaemia is often incompletely understood and inadequately treated. This research topic collates various manuscripts along diverse strands but is unified on the common theme of hypoglycaemia to enrich our current understanding of disease pathways, therapeutic targets and patient impact centred around patient need (Figure 1).

Kristensen et al. gets to the heart of the health consequences of hypoglycaemia but concludes that Health related Quality of Life (QoL) studies are infrequent, inconsistent in their reports and often generic to chronic illness, thereby failing to capture disease specific nuances and family perspectives, for example in the case of Congenital Hyperinsulinism (CHI), a disease of severe and recurrent hypoglycaemia in early childhood. The present generation of generic instruments are not sensitive to individual family distress and are therefore unlikely to capture everyday parental anxiety and feelings of despair and helplessness. There is a case to develop CHI specific QoL instruments and apply these to individual aetiologies to draw out meaningful change and derive valid comparisons.

Genetic technology has changed rapidly over the past decade and genetic investigation of hypoglycaemia is no exception. Maiorana et al. discuss gene panel testing in the context of metabolic causes of hypoglycaemia whilst including hyperinsulinism and CHI. Delineating specific genetic association or causation is important to provide certainty of diagnosis without complex and often uncertain biochemical investigations for optimal pharmacologic therapy and for anticipation of prognosis or disease behaviour. Hewat et al. also round up various genetic aetiologies for CHI but suggest caution in simplistic interpretation; phenotypes are variable and a genetic reporting strategy based on previously recognized gene variants may fail to account for the wide variety of seemingly disparate genetic aetiologies and ignore nuances in appreciating pathogenic behaviour. The authors argue for an informed genetic investigational approach utilizing the experience and interpretive abilities of well-established and research active genetic laboratories.

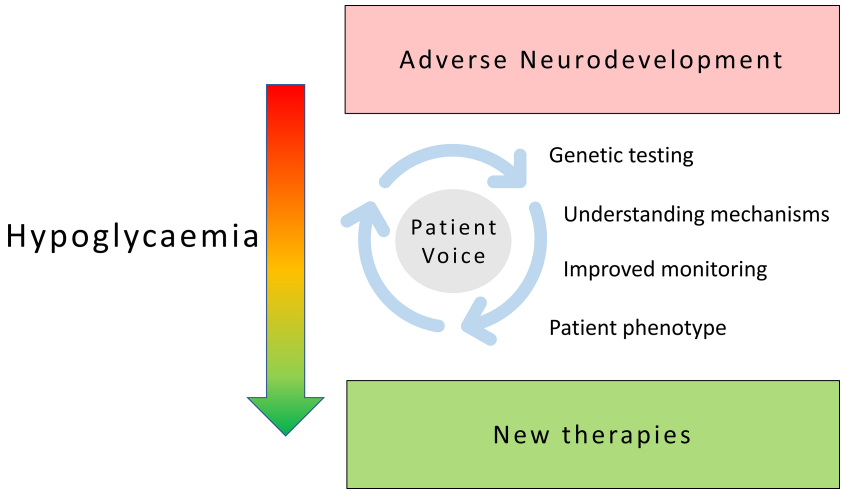

FIGURE 1
Patient focused research in childhood hypoglycaemia: shifting the burden of neurodisability in childhood hypoglycaemia to new therapies and improved outcomes requires sustained exploration of diverse themes centred around an active patient voice.

Understanding of genetic aetiology is important not just for the diagnosis of specific forms of hypoglycaemia, such as focal CHI but also for dissecting syndromic forms of CHI described by Zenker at al. The authors explore the strength of association, some being tenuous while others having closer correlation and some having distinctive pancreatic histopathological changes, for example those with Beckwith Wiedemann syndrome. The diversity of genetic aetiology reiterates the heterogeneity of hyperinsulinism in CHI in a nod to Wieland et al. who question traditional understanding of differential gene expressions in the well-recognized form of focal CHI. The authors explore the possibility of integrating pancreatic gene transcript expression to enrich understanding of focal lesional pathobiology. While the recognition of novel genetic correlates may appear to diversify existing heterogeneity, it may promote deeper understanding of pathways for regulation of insulin and potential therapeutic windows in hypoglycaemia.

At present, medical therapies for CHI, a leading cause of childhood hypoglycaemia, are suboptimal; the mainstay of CHI therapy with diazoxide is not applicable to those with mutations in the ATP sensitive K^+ channel (K-ATP) targeted by diazoxide. van Albada et al. describe alternative therapeutic paradigms in the somatostatin pathway regulating insulin secretion. While somatostatin receptor activation may modulate excess insulin in patients with severe genetic forms of CHI, therapy remains off-label both for short and long-acting forms, although with the promise of newer therapies targeting somatostatin receptor subtypes.

Glucagon like peptide 1 (GLP1) as another molecular target and pathway in CHI amenable to therapeutic modulation, has been reviewed by Danowitz and de Leon. The authors describe the physiology of GLP1 and a similar peptide glucose-dependent insulinotropic polypeptide (GIP), setting the scene for potential therapy with GLP1 receptor antagonists for CHI and post prandial forms of hyperinsulinism. It remains to be seen if such insulin modulating mechanisms have sufficient disease modifying ability to enter the hypoglycaemia treatment repertoire.

A radical departure from traditional investigational methods is described in a paper by Lithovius and Otonkoski. The authors discuss current use of pluripotent stem cells (PSCs) with predicted technical advances leading to the conversion of differentiated PSCs into pancreatic islets to serve as a paradigm changing model exploring novel mechanisms, beta-cell glucotoxicity and novel therapies. While stem cell technologies emanating from research in hyperinsulinism may benefit the understanding of CHI in the first instance, it is likely that collateral information will strengthen our understanding of beta-cell biology and have wider application in other beta-cell disorders such as diabetes.

While molecular technologies advance at pace providing exciting new aetiologies of hypoglycaemia, phenotypic exploration using current investigational tools must also continue, if only to understand the link between abnormal genetic mechanisms and disease manifestations. In the paper by Rossi et al. dynamic methods to characterize hypoglycaemia have been discussed. These range from traditional techniques such as functional *in vivo* testing to more modern *in vivo* metabolic profiling using Continuous Glucose Monitoring (CGM). The paper also discusses the value of isotope tracing to dissect aetiologies in inherited errors of metabolism through the interrogation of metabolites and pathways, for instance in glycogen storage disorder type 1 (GSD1).

There is no substitute to natural history studies in hypoglycaemia, mirroring real-world patient information. Although imperfect with inherent data variability, natural history studies of hypoglycaemia captured in global registries are valuable sources of information to design clinical studies and novel therapies. The topic features a narrative of the development of registries in rare diseases as a whole (Kolker et al.) and more specifically in CHI (Pasquini et al.), a hypoglycaemia disorder with

significant family impact. Patient reported data in carefully curated registries are excellent platforms to characterize phenotypic detail that supplement clinician gathered information. As such datasets mushroom, encompassing different groups of patients in different countries, critical evaluation of disease trajectories will no doubt lead to cross correlation of multi-sourced information to build a multidimensional, enriched and individualized model of health need.

A crucial component of the health need in hypopglycaemia is the patient voice, which is often neglected in the face of rapidly expanding technologies and medical opinions. Auckburally et al. take up the patient side to explore the often-unheard voices concurring and dissenting in the use of rapidly emerging technologies such as CGM in hypoglycaemia. Their focused thematic analysis provides a clarion call to adapt and innovate on patient need for optimal health outcomes tailored to the individual. Their manuscript reinforces the increasing importance of the patient perspective in the design of studies and clinical services as joint collaborative enterprises between different disciplines and patient groups, all working harmoniously towards a common goal to alleviate morbidity from debilitating hypoglycaemia.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

IB and KM have been Principle Investigators or Co-investigators in clinical trials sponsored by pharmaceuticals in the management of hypoglycaemia disorders Zealand, Hanmi, Rezolute. IB has been funded to undertake a study of monitoring of hypoglycaemia by Crinetics Pharmaceuticals. The authors do not have direct financial conflict in relation to the submission of this editorial.

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Health-Related Quality of Life of Children and Adolescents With Congenital Hyperinsulinism – A Scoping Review

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Introduction: Despite improvements in diagnosis and therapeutic advances in treatment, congenital hyperinsulinism (CHI) remains a severe disease with high patient impairment. We aimed to review the literature on Health-related Quality of Life in children and adolescents with congenital hyperinsulinism and summarize the findings.

Materials and Methods: For this scoping review, a literature search was conducted in PubMed and Web of Science in May 2021. Inclusion and exclusion criteria for the selection of articles were defined a priori.

Results: Two hundred and forty-five (245) articles were identified through the search and screened on the basis of title and abstract. The full texts of forty articles were then assessed. Finally, four articles (published 2012-2020) describing Health-related Quality of Life in children and adolescents with congenital hyperinsulinism were included. The study designs were heterogeneous and included cross-sectional observational studies (n=2), clinical trials (n =1), and case reports (n=1) with different sample sizes. Three studies were conducted in European countries and one in Japan. The results for Health-related Quality of Life revealed inconsistencies.

Conclusion: There are only a few studies looking at Health-related Quality of Life in children and adolescents with congenital hyperinsulinism. To gain a comprehensive understanding of the impact of congenital hyperinsulinism on Health-related Quality of Life in children and adolescents, it is necessary to use both generic and condition-specific instruments to measure Health-related Quality of Life of young patients in larger samples, to collect longitudinal data, and to consider qualitative research approaches.

Keywords: Health-related quality of life, congenital hyperinsulinism, children, adolescents, patient-reported outcomes

INTRODUCTION

Congenital hyperinsulinism (CHI) is a heterogeneous disorder that results in excessive, often unregulated, insulin secretion from pancreatic beta cells. Clinical manifestations range from life-threatening hypoglycaemia in newborns to mild symptomatic hypoglycaemia in early childhood, adolescence or adulthood, which can be difficult to identify (1).

Although CHI is a rare chronic health condition, it is the most common form of severe recurrent hypoglycaemia in infancy and childhood (1).

Due to the combination of hypoglycaemia and hypoketonaemia at an early age when neurons and neuronal networks are vulnerable to metabolic maladaptations, CHI is associated with abnormal neurodevelopmental outcomes with a tendency toward motor and language delays in early childhood (2). Incidence rates of abnormal neurodevelopmental outcomes varying between 26 and 44% have been reported for CHI patients (2, 3).

Also, the treatment can be challenging for patients. Careful glucose monitoring and daily oral or subcutaneous medication are strategies to manage CHI (4). However, oral diazoxide, the only drug for long-term treatment of hyperinsulinemic hypoglycaemia approved by the Food and Drug Administration (FDA), can lead to varying side effects such as excess hair growth, poor appetite, and fluid retention (5). Dietary management may also be required for reliable glucose delivery and the prevention of hypoglycaemia. However, feeding problems such as difficulty in swallowing, vomiting, and refusal to eat can complicate the management of hypoglycaemia, making tube feeding necessary (6).

When CHI is diagnosed, and normoglycaemia is achieved quickly, most children develop a normal range of cognitive, emotional, and social abilities. In these cases, complete clinical remission has been observed in medically responsive patients after several years of intensive conservative treatment (7).

A near-total pancreatectomy may be necessary for the most severe diffuse forms of CHI, in which abnormal beta cells are distributed throughout the pancreas and cannot be treated successfully with medication. However, as the size of the resection increases, the risk of surgery-related sequelae increases, particularly the possible development of pancreatic exocrine dysfunction, persistent hyperglycaemia or insulin-dependent diabetes. These complications of surgical therapy can also be observed with a latency of several years, e.g. with the onset of puberty (8).

Due to the above described consequences, both disease and treatment related, CHI may have a substantial impact on the Health-related Quality of Life (HrQoL) of those affected. The term HrQoL is a subjective, self-assessed and multidimensional construct that describes a person's perception of his or her own physical, psychological, and social health status (9). This includes the impact of illness and treatment on the perception of health.

In the absence of a review on HrQoL in children and adolescents with CHI, this article aims to provide an overview of the current literature with the aim to improve our understanding of HrQoL in young CHI patients and to enhance the appropriate use of HrQoL instruments in research and practice.

METHODS

Search Strategy and Eligibility Criteria

On May 19, 2021 we conducted a literature search in the databases PubMed and Web of Science which we updated it until September 23, 2021. The methodological framework of Arksey and O'Malley's five-stage scoping review formed the basis for the literature search approach (10).

This review considers both qualitative and quantitative studies which focus on HrQoL in children and adolescents (aged 0 to 18 years) diagnosed with CHI. A prerequisite for inclusion in the review is that HrQoL was measured. The study design is therefore considered secondary as long as HrQoL was measured quantitatively or qualitatively in the studies. Thus, the review finds empirical studies such as cross-sectional studies, longitudinal studies, clinical trials, case reports or case series, and meta-analyses. No geographical, language, or time restrictions were placed. However, only studies that are published in peer-reviewed journals are included. These inclusion and exclusion criteria were defined a priori.

Since HrQoL is sometimes confused with or used as a synonym for other constructs, terms such as mental health, psychosocial health, and well-being were also considered. The complete search strategy included a combination of keywords and MeSH terms: ("Congenital Hyperinsulinism" [Mesh] OR "congenital hyperinsulinism") AND (child* OR youth* OR teen* OR adolescent* OR infant* OR juvenile) AND ("quality of life" [Mesh] OR well-being OR psychosocial OR "mental health" OR "quality of life").

Selection Process and Data Extraction

After conducting the database search, exporting results, and removing duplicates, two independent reviewers screened titles and abstracts. The full texts of the articles that met the eligibility criteria or the articles for which it was not possible to assess eligibility based on the title and abstract were retrieved for further assessment. The same two independent reviewers evaluated all full-text articles against eligibility criteria. Any disagreements or uncertainties about eligibility were discussed until a consensus was reached.

A data extraction form was used for the final set of included studies: study population and setting; study design; the number of participants; participant characteristics; outcome measure; and the main results.

RESULTS

Study Selection

The database search yielded a total of 245 citations. After title and abstract screening, 40 full texts were reviewed, of which four met the eligibility criteria and were included (11–14). The study

selection process is shown in **Figure 1**, which is based on the PRISMA Statement (15).

Study Characteristics

The four studies included in this scoping review were published between 2012 and 2020 (11, 14). They were mainly conducted in Europe (Finland, France, UK) (11–13) with the exception of Japan (14). Study designs include one cross sectional and one retrospective survey (12, 14), a longitudinal observational study (11), and a case report (13). The sample sizes ranged between one participant (13) and 447 participants (14). Instruments used to measure HrQoL of children and adolescents with CHI were the generic patient-reported outcome measures (PROMs) KINDL-R (12), PedsQL (13), AUQUEI picture questionnaire, and the QUALIN questionnaire (11) as well as a physician-reported questionnaire (14). A summary of study characteristics is provided in **Table 1**.

HrQoL in Children and Adolescents Diagnosed With CHI

In total, two studies were of an observational nature, i.e. aimed at describing HrQoL of children and adolescents with CHI. Männistö and colleagues investigated the impact of transient (n=32) and persistent (n=33) CHI on the HrQoL of 3–17-year-old patients recruited from the Finnish CHI registry (12). Transient CHI was defined as a neonatal onset of hyperinsulinism followed by successful discontinuation of medication within four months. Parents were asked to rate their children's HrQoL using the revised KINDL-R. Children and adolescents aged 11 years and older were also given a self-report version of the questionnaire. The results showed that there were no notable differences between the two groups. However, differences were found when compared to the age- and gender-specific reference values of the instrument. In the persistent CHI group, the scores were significantly higher in self-reports in the dimensions physical well-being ($p < 0.001$), self-esteem ($p=0.002$), and total scores ($p=0.038$). In contrast, self-esteem ($p=0.021$) was rated higher in parent reports. The scores of the transient CHI group were statistically higher in self-reported school-related well-being ($p=0.032$). In comparison, scores were higher in physical well-being ($p<0.001$) and total scores ($p=0.013$) in parent reports. The authors note that the results reflect improved management of recently born and treated CHI patients, as none of the included patients underwent subtotal pancreatectomy, which is associated with a high risk of insulin-dependent diabetes and pancreatic exocrine dysfunction, and none of the patients had an intellectual disability due to a severe hypoglycaemic insult. Furthermore, results might reflect the high number of patients who were able to discontinue treatment.

Yamada et al. chose a different data collection strategy (14). In a nationwide survey of clinics, those who had treated patients with endogenous hyperinsulinemic hypoglycaemia between 2017 and 2018 were asked to characterize these patients in more detail. In total, 447 patients with CHI were identified in this survey [transient CHI (n = 197), persistent CHI (n = 225), and unknown subtype (n = 25)]. The questionnaire concerning CHI patients included questions on socio-demographic information, treatment details, and post-treatment outcomes, including HrQoL. However, it was not explained how the external assessment of HrQoL was obtained. A detailed description of the HrQoL of CHI patients was not provided to the readers, only the statement that HrQoL was frequently impaired post-treatment in patients with endogenous hyperinsulinemic hypoglycaemia.

Two studies had a more experimental approach. Le Quan Sang et al. reported outcomes for ten children aged one to eight years, in whom the three daily subcutaneous octreotide injections were replaced by a single and monthly intramuscular injection of long-acting release (LAR) octreotide (11). The participants' HrQoL was assessed using the AUQUEI picture questionnaire for children aged three to eight years and the QUALIN parental report questionnaire. HrQoL was measured at the first injection visit and six months later. No change in either child-reported or parent-reported HrQoL was found in this period. However, satisfaction with the new treatment option was rated high by all parents.

In their case report, Shah and colleagues described how HrQoL of a 14-year-old patient improved significantly after one year of treatment with subcutaneous injections of long-acting somatostatin analogue compared to the previous treatment with orally administered diazoxide (13). In addition to the PROM PedsQL reported by the parents and child, a semi-structured interview was conducted with the family. The patient reported that she was bullied during diazoxide treatment because of the excessive hair growth it caused and that her adherence to the treatment was poor as a result of that. With the switch to monthly injection of LAR octreotide, these side effects disappeared and the patient became more satisfied overall. Both the parents and the patient reported a significant improvement in HrQoL after one year on the new treatment.

DISCUSSION

This scoping review identified four articles describing HrQoL in children and adolescents diagnosed with CHI. Only one study compared HrQoL scores with reference values. Two other studies addressed the change in HrQoL resulting from new treatment procedures. Furthermore, the fourth study described the retrospective physician reported post-treatment HrQoL of CHI patients treated for hypoglycaemia in hospitals. The results described are inconclusive; however, they are difficult to compare due to their different study designs and the different methods of measuring HrQoL.

The compilation of studies illustrates that research on HrQoL in patients with CHI is still in its early stages. More observational studies with larger sample sizes and longitudinal designs are needed to profoundly understand whether and how CHI affects HrQoL in children and adolescents. In this context, more detailed insights, e.g., the extent to which disease activity and adaption to the condition impacts HrQoL in patients with CHI, should also be investigated in more detail. Validated PROMs should be used for this purpose. The identified HrQoL studies

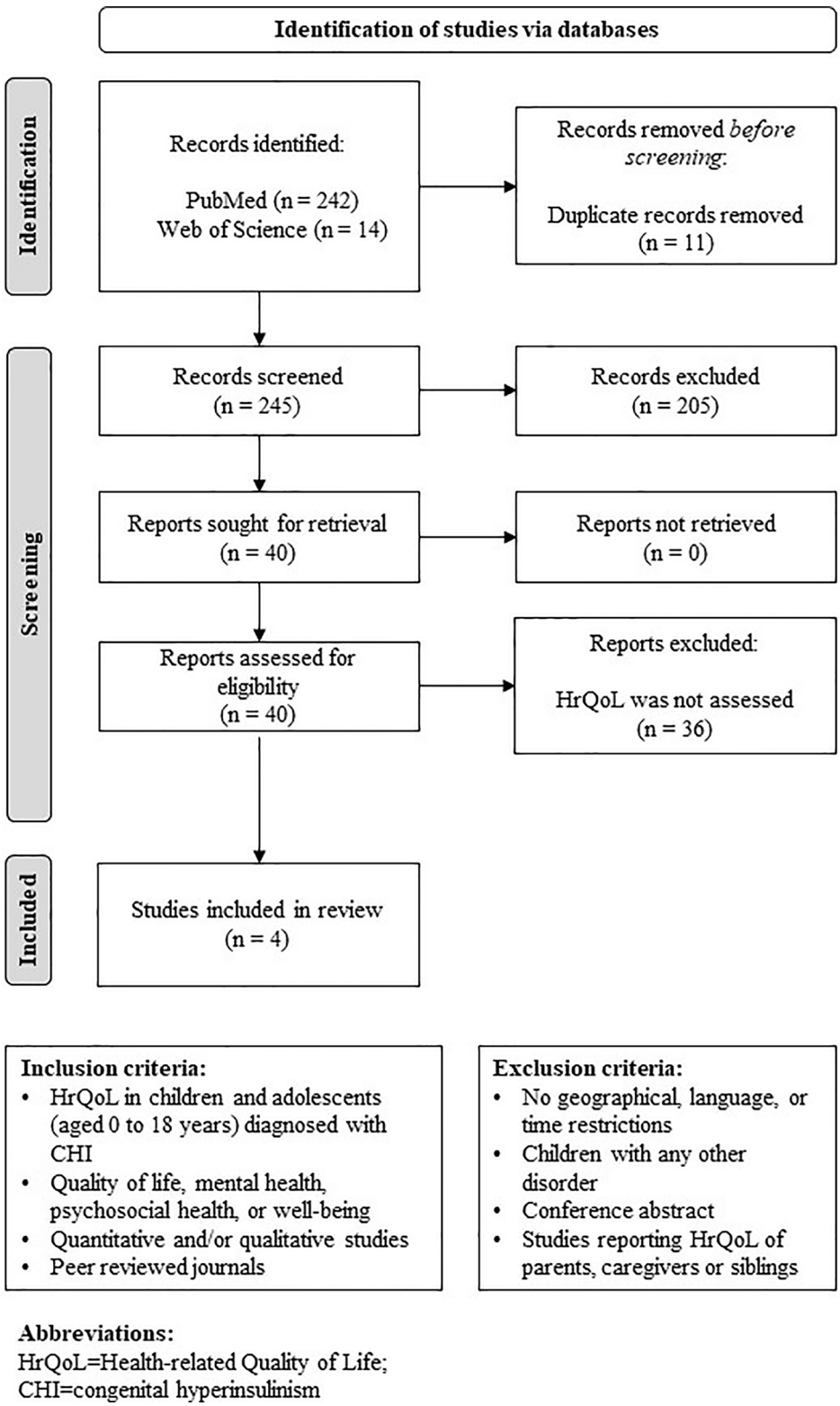

FIGURE 1 | PRISMA flowchart of study selection and inclusion process.

TABLE 1 | Summary of included studies.

Author; Country	Aim	Study Design	Study Population	HrQoL Measures	Results
Le Quan Sang et al. (11); France	To describe changes in various outcomes after replacing three daily octreotide injections by a monthly injection of long-acting release octreotide in CHI patients	clinical trial, pre-post	n = 10; age: 1-8 years	Child-report: AUQUEI; Parent-report: QUALIN	A monthly injection of LAR octreotide was efficient in maintaining glycaemia unchanged, without altering the normal weight-and-growth.The HrQoL evaluations of the children and parents were not able to detect any change after the switch of the new treatment (AUQUEI M=8.0, SD=1.33 at six months vs. M=7.9, SD=1.45 at baseline).
Männistö et al. (12); Finnland	To examine whether the HRQoL is worsened in patients with persistent or transient CHI.	cross-sectional survey	Parent-report: n = 65; child-report: n = 19; age: 3-17 years	KINDL, child-report, parent-report	In self-reports of subjects aged 11–17 years and in parent reports of children aged 3–17 years, Persitant-CHI or Transient-CHI children did not have statistically lower scores in any of the six dimensions (physical well-being, emotional well-being, self-esteem, family, friends, and school) or in total scores compared to the reference values.
Yamada et al. (14); Japan	To investigate the incidence, treatment details and outcomes of patients with endogenous hyperinsulinemic hypoglycemia (EHH), including those with transient/persistent CHI.	Retrospective survey	n = 447 patients with CHI	Physician-reported retrospective HrQoL	Findings indicated an improvement in the prognosis of persistent CHI over the past 10 years. However, frequent post-treatment residual hypoglycemia and impaired quality of life were reported too.
Shah et al. (13); United Kingdom	To report the first case on the use of lanreotide in an adolescent girl with diazoxide-responsive CHI.	case report, pre post design	n = 1; age: 14 yreas	PedsQL, child-report, parent-report	On Diazoxide, both parents and child reported a clinically significantly lower HrQoL with respect to psychosocial aspects in the PedsQL There was a significant improvement in HrQoL after 1 year on Long-Acting Somatostatin Analogue

AUQUEI, Pictured Child's Quality of Life Self Questionnaire; KINDL-R, Revised questionnaire to assess Health-Related Quality of Life in children and adolescents; PedsQL, Pediatric Quality of Life Inventory; CHI, Congenital hyperinsulinism; n, number; QUALIN, Infant Quality of Life; HrQoL, Health-related Quality of Life; p, significance.

used validated generic HrQoL instruments, specifically, the KINDL-R, PedsQL, QUALIN, and AUQUEI questionnaires in three out of four studies. The advantage of these instruments is that comparisons with the general population or populations affected by other health conditions are possible (16).

Regarding clinical research, it should be noted that HrQoL played a rather minor role in the clinical trials we identified in our search. In one study, HrQoL was included as a secondary outcome in a clinical trial with a pre-post study design (11). As noted in our literature search, clinical outcomes, such as glycaemias or neurodevelopmental outcomes, have been studied more frequently. However, there is no question that PROMs are also essential in clinical research. In particular, where evidence of economic benefit is critical to the success of orphan drug reimbursement, robust PROM data are crucial (17).

PROMs were initially used at an aggregate level, as in the observational and clinical studies identified in this review. However, PROMs are also becoming increasingly important in clinical practice. By asking routine screening questions, physicians can use the results of PROMs as a basis for the consultation, which can help improve communication between physicians and patients (18). Moreover, PROMs can be collected on an ongoing basis to monitor patient progress and identify problems such as disease progression and possible side effects of prescribed treatments. Studies have shown that the routine use of PROMs not only increases patient satisfaction but can also improve patient outcomes, including symptom control and HrQoL (19, 20).

When considering the use of HrQoL in clinical trials or in clinical practice, it is important to be aware of the different attributes of PROMs. Especially for rare diseases, condition specific PROMs can be helpful. Compared to generic PROMs, they address the most specific concerns of patient populations and are more sensitive to condition-specific changes (16). To date, there is no CHI-specific instrument to measure HrQoL. Given the development of novel medical therapies such as LAR octreotide injections and also surgical advances such as laparoscopic pancreatectomy (21), the development of a CHI-specific HrQoL instrument would be an asset to the evaluation of these novel interventions.

In order to develop a CHI-specific HrQoL instrument, qualitative research is needed. By giving patients a voice, it becomes possible to understand the patients experience in depth and to capture the full range of manifestations and their relationship to HrQoL. In one case report included in this review, a semi structured interview was conducted with the adolescent patient and her parents (13). Valuable insights emerged from the interview, such as how the excessive hair growth caused by diazoxide treatment was the reason she was bullied by other children and led to poor treatment adherence. It is testimonials like these, after all, excessive hair growth is a common side effect of diazoxide (21), that are essential for characterizing the disease-specific HrQoL. However, this should be done in a systematic way with clearly defined research questions.

However, there are several issues to consider when developing a CHI-specific HrQoL instrument. The rarity of the condition is a challenge for the development and validation of a PROM. Cost-intensive international studies can increase the sample size needed for validation, but linguistic and cultural differences must be considered (22). At the same time, the heterogeneity

of CHI manifestation should be taken into account. Stratification according to age groups could be a helpful strategy. Due to the young age of the patients, parents' perspective should also be included (23), as was the case in the reviewed studies. For the development of a PROM, which should be preceded by a thorough systematic literature review, the identification of existing PROMs for patients with similar symptoms, such as type 1 diabetes-specific PROMs for CHI patients who have developed insulin-dependent diabetes, could serve as a starting point.

It should be noted that this is a scoping review that does not claim to identify all existing studies on HrQoL in children and adolescents with CHI. It allows us to summarize research findings, draw conclusions from the existing literature about the general state of research activity, and identify gaps in the evidence base where research has not yet been conducted. However, it is essential to bear in mind that quality assessment does not form part of a scoping study (10). Therefore, it is not possible to identify gaps in the literature that are due to poor research quality.

In summary, few studies to date have addressed HrQoL in children and adolescents with CHI. The few studies that do exist yield inconsistent results while different study designs hamper comparisons. More observational studies characterizing HrQoL with mixed-methods approaches are needed. The use of validated generic and condition-specific HrQoL instruments can not only contribute to a deeper understanding of HrQoL of patients but also strengthen clinical research.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

KK, SW, and JQ developed the study concept and the design. KK and SW screened the publications and identified relevant articles. KK wrote the first draft of the manuscript. SW and JQ revised the first draft critically for important intellectual content. All authors have revised the subsequent drafts critically and agreed to be accountable for all aspects of the work. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Worth C, Yau D, Salomon Estebanez M, O'Shea E, Cosgrove K, Dunne M, et al. Complexities in the Medical Management of Hypoglycaemia Due to Congenital Hyperinsulinism. *Clin Endocrinol* (2020) 92:387–95. doi: 10.1111/cen.14152
2. Ludwig A, Ziegenhorn K, Empting S, Meissner T, Marquard J, Holl R, et al. Glucose Metabolism and Neurological Outcome in Congenital Hyperinsulinism. *Semin Pediatr Surg* (2011) 20:45–9. doi: 10.1053/j.sempedsurg.2010.10.005
3. Meissner T, Wendel U, Burgard P, Schaetzle S, Mayatepek E. Long-Term Follow-Up of 114 Patients With Congenital Hyperinsulinism. *Eur J Endocrinol* (2003) 149:43–51. doi: 10.1530/eje.0.1490043
4. Yorifuji T. Congenital Hyperinsulinism: Current Status and Future Perspectives. *Ann Pediatr Endocrinol Metab* (2014) 19:57–68. doi: 10.6065/apem.2014.19.2.57
5. Welters A, Lerch C, Kummer S, Marquard J, Salgin B, Mayatepek E, et al. Long-Term Medical Treatment in Congenital Hyperinsulinism: A Descriptive Analysis in a Large Cohort of Patients From Different Clinical Centers. *Orphanet J Rare Dis* (2015) 10:150. doi: 10.1186/s13023-015-0367-x
6. Banerjee I, Forsythe L, Skae M, Avatapalle HB, Rigby L, Bowden LE, et al. Feeding Problems Are Persistent in Children With Severe Congenital Hyperinsulinism. *Front Endocrinol* (2016) 7:8. doi: 10.3389/fendo.2016.00008
7. Lachmann MJ, Salgin B, Kummer S, Welters A, Döing C, Zenker M, et al. Remission of Congenital Hyperinsulinism Following Conservative Treatment: An Exploratory Study in Patients With KATP Channel Mutations. *J Pediatr Endocrinol Metab JPEM* (2016) 29:281–7. doi: 10.1515/jpem-2015-0192
8. Meissner T, Mayatepek E. Kongenitaler Hyperinsulinismus. *Monatsschrift Kinderheilkunde* (2005) 153:483–94. doi: 10.1007/s00112-005-1129-y
9. Chassany O, Sagnier P, Marquis P, Fullerton S, Aaronson N. Patient-Reported Outcomes: The Example of Health-Related Quality of Life—A European Guidance Document for the Improved Integration of Health-Related Quality of Life Assessment in the Drug Regulatory Process. *Drug Inf J* (2002) 36:209–38. doi: 10.1177/009286150203600127
10. Arksey H, O'Malley L. Scoping Studies: Towards a Methodological Framework. *Int J Soc Res Methodol: Theory Pract* (2005) 8:19–32. doi: 10.1080/1364557032000119616
11. Le Quan Sang KH, Arnoux JB, Mamoune A, Saint-Martin C, Bellanné-Chantelot C, Valayannopoulos V, et al. Successful Treatment of Congenital Hyperinsulinism With Long-Acting Release Octreotide. *Eur J Endocrinol* (2012) 166:333–9. doi: 10.1530/EJE-11-0874
12. Männistö JME, Jääskeläinen J, Huopio H. Health-Related Quality of Life in Children With Congenital Hyperinsulinism. *Front Endocrinol* (2019) 10:670. doi: 10.3389/fendo.2019.00670
13. Shah P, Rahman SA, McElroy S, Gilbert C, Morgan K, Hinchey L, et al. Use of Long-Acting Somatostatin Analogue (Lanreotide) in an Adolescent With Diazoxide-Responsive Congenital Hyperinsulinism and Its Psychological Impact. *Hormone Res Paediatr* (2015) 84:355–60. doi: 10.1159/000439131
14. Yamada Y, Kitayama K, Oyachi M, Higuchi S, Kawakita R, Kanamori Y, et al. Nationwide Survey of Endogenous Hyperinsulinemic Hypoglycemia in Japan (2017-2018): Congenital Hyperinsulinism, Insulinoma, Non-Insulinoma Pancreatogenous Hypoglycemia Syndrome and Insulin Autoimmune Syndrome (Hirata's Disease). *J Diabetes Invest* (2020) 11:554–63. doi: 10.1111/jdi.13180
15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 Statement: An Updated Guideline for Reporting Systematic Reviews. *BMJ* (2021) 372:n71. doi: 10.1136/bmj.n71
16. Benjamin K, Vernon MK, Patrick DL, Perfetto E, Nestler-Parr S, Burke L. Patient-Reported Outcome and Observer-Reported Outcome Assessment in Rare Disease Clinical Trials: An ISPOR COA Emerging Good Practices Task Force Report. *Value Health* (2017) 20:838–55. doi: 10.1016/j.jval.2017.05.015
17. Lyons EJ, McGeagh J, Es-Skali IJ, Parmenter L, Nijhuis T, Boucher G. The Importance of Patient Reported Outcomes in Reimbursement of Orphan Products in Europe. *Value Health* (2013) 16:A326. doi: 10.1016/j.jval.2013.08.021
18. Velikova G, Booth L, Smith AB, Brown PM, Lynch P, Brown JM, et al. Measuring Quality of Life in Routine Oncology Practice Improves Communication and Patient Well-Being: A Randomized Controlled Trial. *J Clin Oncol* (2004) 22:714–24. doi: 10.1200/JCO.2004.06.078

19. Graupner C, Kimman ML, Mul S, Slok AHM, Claessens D, Kleijnen J, et al. Patient Outcomes, Patient Experiences and Process Indicators Associated With the Routine Use of Patient-Reported Outcome Measures (PROMs) in Cancer Care: A Systematic Review. *Supportive Care Cancer* (2021) 29:573–93. doi: 10.1007/s00520-020-05695-4
20. Lapin BR, Honomichl RD, Thompson NR, Rose S, Sugano D, Udeh B, et al. Association Between Patient Experience With Patient-Reported Outcome Measurements and Overall Satisfaction With Care in Neurology. *Value Health* (2019) 22:555–63. doi: 10.1016/j.jval.2019.02.007
21. Giri D, Hawton K, Senniappan S. Congenital Hyperinsulinism: Recent Updates on Molecular Mechanisms, Diagnosis and Management. *J Pediatr Endocrinol Metab JPEM* (2021) jpem-2021-0369. doi: 10.1515/jpem-2021-0369
22. Wild D, Grove A, Martin M, Eremenco S, McElroy S, Verjee-Lorenz A, et al. Principles of Good Practice for the Translation and Cultural Adaptation Process for Patient-Reported Outcomes (PRO) Measures: Report of the ISPOR Task Force for Translation and Cultural Adaptation. *Value Health J Int Soc Pharmacoeconomics Outcomes Res* (2005) 8:94–104. doi: 10.1111/j.1524-4733.2005.04054.x
23. Matza LS, Patrick DL, Riley AW, Alexander JJ, Rajmil L, Pleil AM, et al. Pediatric Patient-Reported Outcome Instruments for Research to Support Medical Product Labeling: Report of the ISPOR PRO Good Research Practices for the Assessment of Children and Adolescents Task Force. *Value Health J Int Soc Pharmacoeconomics Outcomes Res* (2013) 16:461–79. doi: 10.1016/j.jval.2013.04.004

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Stem Cell Based Models in Congenital Hyperinsulinism – Perspective on Practicalities and Possibilities

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Congenital hyperinsulinism (CHI) is a severe inherited neonatal disorder characterized by inappropriate insulin secretion caused by genetic defects of the pancreatic beta cells. Several open questions remain in CHI research, such as the optimal treatment for the most common type of CHI, caused by mutations in the genes encoding ATP-sensitive potassium channels, and the molecular mechanisms of newly identified CHI genes. Answering these questions requires robust preclinical models, particularly since primary patient material is extremely scarce and accurate animal models are not available. In this short review, we explain why pluripotent stem cell derived islets present an attractive solution to these issues and outline the current progress in stem-cell based modeling of CHI. Stem cell derived islets enable the study of molecular mechanisms of CHI and the discovery of novel antihypoglycemic drugs, while also providing a valuable model to study the biology of variable functional states of beta cells.

Keywords: congenital hyperinsulinism, stem cell derived islets, disease modeling, drug screening and discovery, insulin secretion, hypoglycemia, genetic defects

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INTRODUCTION

Congenital hyperinsulinism (CHI), characterized by inappropriate insulin secretion from the pancreatic beta cells, is the most common cause of persistent childhood hypoglycemia. At least 15 causative genes have been identified (1), with 30-55% of patients remaining without a genetic diagnosis (2–5). Over 50% of all CHI patients (2, 5) carry a recessive loss-of-function mutation in the K_{ATP}-channel genes *ABCC8* or *KCNJ11* (K_{ATP}HI), which leads to abnormal membrane depolarization and constitutive insulin secretion. Clinically, this leads to severe hypoglycemia for which there is no optimal treatment. This represents an ongoing clinical challenge as the hypoglycemia is life-threatening in the first days of life and despite best contemporary treatment, many continue to suffer from learning difficulties in the long term (6–10). A robust preclinical model would be required for studies aiming to discover improved treatment options for K_{ATP}HI and to pinpoint molecular mechanisms of rare newly identified forms of CHI.

Pluripotent stem cells (PSCs) represent the epiblast cells of the early embryo, capable of differentiation to any cell type in the human body. Tissue differentiated from PSCs holds enormous

promise in regenerative medicine to replace or repair a damaged or degenerated organ. PSCs can also serve as a powerful research tool by allowing limitless generation of difficult-to-procure tissue and would thus serve as an attractive solution for preclinical study of CHI. Pluripotent stem cell derived islets (SC-islets) could replace or complement rodent models and primary patient islet tissue, which both have important inherent weaknesses. Rodent islets are structurally and physiologically different from human islets (11–14) and these differences have manifested in K_{ATP}-channel knockout mouse models, which have presented with a much milder phenotype than the K_{ATP}HI patients (15–17). The availability of healthy primary islets is limited and faces issues of variable *in vitro* function (www.epicore.ualberta.ca/isletcore/). The availability of CHI patient islets presents an additional challenge due to the rarity of the disease. The limited tissue availability challenges any study that requires large amounts of tissue, such as screening for novel pharmacotherapeutics.

This review focuses on the use of PSC-derived pancreatic islets (SC-islets) for preclinical study of congenital hyperinsulinism. We outline the practical necessities in setting up SC-islet models and aim to identify relevant questions for CHI research where the SC-islets are particularly powerful.

SC-ISLET BASED DISEASE MODELING: KEY TECHNOLOGIES AND CURRENT PROGRESS

Genome Editing of Pluripotent Stem Cells

Pluripotent stem cells (PSCs) are derived from two main sources: preimplantation embryos (embryonic stem cells, ESCs) (18) and somatic cells that have been reprogrammed back to pluripotent state by overexpression of key genes (induced pluripotent stem cells, iPSCs) (19). iPSCs reprogrammed from a patient sample carry the disease-causing mutations of that individual and should thus phenocopy the disease, such as CHI, when differentiated. A similarly differentiated healthy iPSC line would serve as a non-isogenic control for this type of approach. Theoretically, this offers a disease model without the need for genome editing. In practice however, the differences in the donor genetic background exert a high degree of influence on the differentiation efficiency of stem cell lines, at least in islet differentiation, making it difficult to conclude whether the detected differences between patient and healthy cell lines are due to the disease gene or a differentiation-related artefact. Thus, it is often more practical to correct the disease-causing mutation with genome editing tools to yield an isogenic control cell line. Isogenic controls offer a clean look into the disease phenotype without differences in the genetic background. Generating isogenic controls with genome editing of the PSCs can be considered as the ideal approach for the effective modeling of a genetic disease such as CHI.

Another approach to create isogenic cell lines is to engineer the disease-causing mutation in a healthy hPSC line. This approach is often more straightforward than the patient cell line approach because the differentiation protocol used to derive the SC-islets can be optimized for one cell line and all the interesting mutations can be engineered to it. Furthermore, from the genome editing point of view, it is easier to generate a knock-out than a knock-in. In correcting a patient line, a knock-in is always required, but for generating a disease cell line a simple knock-out is often enough since many diseases are caused by loss-of-function mutations.

Multiple technologies are available for the genome editing itself, but recently CRISPR-based technologies have started to dominate, due to their relative ease and high efficiency. The basic CRISPR system consists of a guide RNA, able to target the editing to a specific locus in the genome; a protein exhibiting nuclease activity such as Cas9 or Cas12a creating a double stand break; and an RNA template containing the mutation-corrected sequence or a sequence knocking out the healthy gene which can be read during homology directed repair of the double strand break. This basic system has been expanded and optimized further in many ways, as reviewed here (20). Regardless of the specifics, genome editing technology has matured to a state where generating disease relevant stem cell lines for further differentiation is practical.

Differentiation of PSCs to Islets

Due to the curative potential of PSCs in cell replacement therapy for insulin deficient diabetes, enormous effort has been expended in developing protocols that can drive PSCs to differentiate *in vitro* to pancreatic islets (SC-islets). Starting with the breakthrough protocol published in 2006, showing for the first time that insulin positive cells can be differentiated from hPSCs through steps mimicking normal development (21), the progress in the field has been rapid. First evidence of glucose-regulated insulin release was provided in 2014 (22, 23), Since then, many further improvements have been made (24–26) and in the past two years the first protocols giving robust, dynamic glucose stimulated insulin secretion have been reported, achieving beta cell maturity at least in terms of insulin secretory function (27–35). This recent progress in the field has identified multiple conditions related to optimal late-stage maturation. These include keeping SC-islets appropriately sized by resizing or by culture format, keeping the proliferation rate low, normoglycemic culture conditions, lack of ALK5-inhibition, addition of WNT4, circadian entrainment, and reducing the number of unwanted cells that might compromise function on the islet level by sorting or by addition of aurora kinase inhibitor.

Given the recapitulation of the adult function in the state-of-the-art protocols and the fact that the protocols used to derive the mature SC-islet use the same signaling cues as the fetal islets during their *in vivo* development, SC-islets represent an excellent avenue for modeling CHI pathophysiology, including both developmental and insulin secretory defects. We have shown that all the main components of the stimulus-secretion coupling machinery of beta cells: metabolic processing of glucose, currents of the critical ion-channels, the insulin-secretion modulating amplifying mechanisms and the exocytosis machinery, are present in SC-islets (30). As most forms of CHI manifest in

the neonatal period, achieving adult-like function might not even be necessary for some study questions. This is exemplified by existing SC-islet models for CHI, which have been successful even using less efficient differentiation protocols, as described in the following section.

Stem Cell-Based Models for CHI

Thus far, two studies have taken advantage of the SC-islet differentiation and genome editing technologies in modeling K_{ATP}-channel related CHI (K_{ATP}HI) (36, 37), as summarized in **Table 1**. Guo and colleagues (36), used healthy hESCs and introduced a knockout of the *ABCC8* gene, encoding the K_{ATP}-channel subunit SUR1. The *ABCC8* KO beta cells secreted around 2-fold more insulin *in vitro* and failed to respond to K_{ATP}-channel acting pharmaceuticals. Their beta-like cells could be inhibited with octreotide, and to a lesser degree with nicorandil and nifedipine. Thus, they replicated the K_{ATP}HI insulin secretion phenotype *in vitro*.

We used iPSCs derived from a patient carrying the homozygous V187D-mutation (38) in the *ABCC8* gene and compared them to mutation-corrected controls (37). The *ABCC8* mutant beta-like cells secreted around 3-fold more insulin in low glucose compared to the corrected counterparts. They also failed to respond to K_{ATP}-channel acting pharmaceuticals but could be inhibited with clonidine and EGTA. Upon transplantation and *in vivo* maturation under the kidney capsule of immunocompromised mice, the mutant grafts secreted 7-fold higher levels of human C-peptide and caused 38% lower blood glucose upon fasting than the control grafts. We could thus replicate the cardinal phenotypic features of K_{ATP}HI both *in vitro* and *in vivo*. In addition to these features of the secretory function, we found that the K_{ATP}-inactivation directed the development of endocrine cells towards beta cells at the expense of alpha cells in the *in vitro* differentiation. This may have been due to the increased proliferation we detected in the *ABCC8* mutant beta cells. The role of K_{ATP}-channel inactivation in proliferation was also found in a previous study using pancreas-derived mesenchymal stem cells derived from a K_{ATP}HI patient (39).

TABLE 1 | Summary of published studies on stem cell based modeling of congenital hyperinsulinism.

***In vitro* phenotype**	K_{ATP}-mutant vs. control
Studies 1&2	
Insulin secretion in low glucose	2-3 fold higher secretion
Response to diazoxide	No response vs. 50% reduction
***In vivo* phenotype**	
Study 2	
Fasting C-peptide	7 fold higher level in circulation
Fasting glucose	40% lower
Developmental phenotype	
Study 2	
Beta cell proportion	32% more beta cells
Beta cell proliferation	61% more proliferating beta cells

Study 1: Guo et al. Scientific Reports (36).
Study 2: Lithovius et al. Diabetologia (37).

POTENTIAL RESEARCH AVENUES FOR SC-ISLET CHI MODELS

Development of Pharmacotherapy for Diazoxide-Resistant CHI

The first-line antihypoglycemic drug used in CHI, diazoxide, is an opener of the K_{ATP}-channel and as such, most of the recessive K_{ATP}HI patients are unresponsive to it (40, 41). Second-line treatments, such as somatostatin receptor agonists are widely used, but still the most severe patients must undergo pancreatectomy to control hypoglycemia. This is a suboptimal treatment which rarely results in euglycemia (42–44). There is an obvious need for more effective antihypoglycemic medication for diazoxide-resistant CHI.

SC-islets can be generated in limitless quantities and when derived with state-of-the-art differentiation protocols (27, 29, 30), they harbor the stimulus-secretion coupling machinery of adult primary islets: the K_{ATP}-channel related triggering pathway, as well as the neurohormonal and metabolic amplifying pathways (30). This covers the key pathways that modulate insulin secretion and could thus serve as pharmacological targets.

Potential antihypoglycemic targets on the beta cell include ion-channels, G-protein coupled receptors and transcription factors controlling beta cell function, as summarized in **Figure 1**. Depending on the genetic cause of CHI, some of these might be more advantageous than others. In the most common type of diazoxide resistant CHI, K_{ATP}HI, insulin hypersecretion occurs because the K_{ATP}-channel is inactive leading to constitutive depolarization and constantly elevated intracellular calcium (45). In this intracellular environment, a molecule acting upstream of the K_{ATP}-channel in the stimulus-secretion coupling machinery (ie. glucose uptake, glycolysis and the TCA cycle) is likely to be ineffective. Potential antihypoglycemic ion-channel acting drugs would act by decreasing the membrane potential independently of the K_{ATP}-channel (ie. by acting on K^+, Na^+ and Cl^- channels) or by reducing intracellular Ca^{2+}, the final trigger of exocytosis. Nifedipine and other Ca^{2+} channel blockers have been used in the treatment of K_{ATP}HI but have proven ineffective (46). Sikimic and colleagues identified DCEBIO, acting as an agonist of the repolarizing $K_{Ca3.1}$ channel, as a molecule abolishing the glucose induced Ca^{2+} oscillations in two K_{ATP}HI islet preparations (47). A potential downside with ion-channel acting molecules is that many of the target channels are also expressed in non-islet cells, such as cardiomyocytes and neurons, increasing the likelihood of serious side effects.

Several islet specific G-protein coupled receptors (GPCRs) have been identified (48). The role of these receptors in beta cells is to fine-tune the glucose-stimulated insulin secretion by affecting the amplifying pathways. They exert their action through cAMP and other second messengers, which sensitize or desensitize insulin granule exocytosis. Human islets express around 300 GPCRs (49). Highly expressed ones include receptors for the other islet hormones, glucagon and somatostatin, and the gut-derived incretins, GLP1 and GIP, but many beta cell GPCRs also have no known ligand (orphan receptors). This offers the potential for novel antihypoglycemic compounds to be found by screening. Many of the most potent GPCR-coupled amplifying pathways,

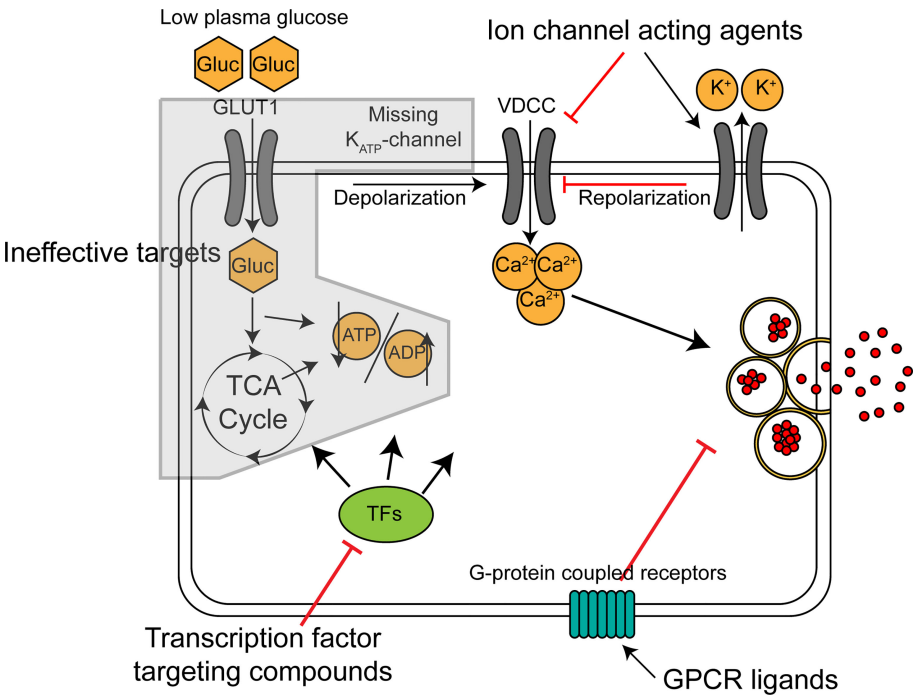

FIGURE 1 | Mechanism of action for classes of potential antihypoglycemic agents for treatment of K_{ATP}-channel related CHI. Broad, schematic depiction of insulin secretion machinery in K_{ATP}-channel deficient beta cells in low glucose. GLUT1, glucose transporter; Gluc, glucose; VDCC, voltage-dependent calcium channel; TCA cycle, tricarboxylic acid cycle; TFs, transcription factors.

such as the GLP1-receptor coupled pathway, are primarily stimulated only when the beta cells are already triggered by the K_{ATP}-channel closure and subsequent influx of calcium, restricting their activity mostly to high glucose conditions (50). The K_{ATP}HI cells are constantly in the state of high $[Ca^{2+}]_i$, which should allow the inappropriate activation of these pathways even in low glucose. Targeting GPCRs in K_{ATP}HI has been shown to be effective by the well-established use of somatostatin receptor agonists such as octreotide. More recently, studies by De León and associates have identified the GLP1-receptor inverse agonist exendin-(9-39) as an effective antihypoglycemic agent in K_{ATP}-KO mice (51) and in adult K_{ATP}HI patients (52).

Transcription factors controlling beta cell insulin secretion could serve as a third group of targets for antihypoglycemic medication. Senniappan and colleagues demonstrated the validity of this approach using mTOR inhibitor rapamycin on diazoxide-resistant CHI patients (53). Since the initial report however, the use of rapamycin has been questioned due to low efficacy and high incidence of serious side effects (54, 55). These issues are likely due to mTOR being an important regulator of wide variety of cellular processes in most tissues, and as such, an agent acting on a transcription factor more specifically controlling beta cell insulin secretion related gene expression would be more ideal. Despite the existence of several beta cell-specific transcription factors that control the expression of insulin secretory machinery genes (e.g., MAFA or RFX6), small molecules specifically targeting them are missing, and thus the ion-channel and GPCR-related strategies are likely to provide more accessible targets.

The most clinically relevant parameter for identifying the potential drug would be its effectiveness in reducing the CHI SC-islets' insulin secretion in low glucose. Measuring just intracellular calcium fluxes with a dye or a genetically encoded sensor could lead to failure in identifying a potential drug if the drug acts on the amplifying mechanisms of insulin secretion, whose activity does not result in further calcium fluxes. Any candidate identified in the *in vitro* screens should be validated *in vivo*. The first line strategy for this is to use CHI SC-islet grafts, which lead to hypoglycemia in the recipient mice (37). Conceivably measurements of mouse blood glucose and C-peptide secreted by the graft in drug-treated and non-treated mice engrafted with CHI SC-islets should reveal the effectiveness of a candidate molecule *in vivo*.

Discovery of Novel CHI Pathomechanisms

Around 50% of new CHI patients do not present a mutation in the genes previously identified as causative for CHI (2). Unraveling of the exact pathogenic mechanism should direct selection of the most appropriate treatment for each patient. Pinpointing the molecular mechanism could also aid discovery of antihypoglycemic pharmaceuticals and shed light to the role of the identified genes in the regulation of beta cell insulin secretion. Answering these questions requires a model system with sufficient fidelity to capture disease pathophysiology on many levels. SC-

islets have been used to discover the molecular mechanism of multiple genes causing different types of monogenic diabetes, ranging from mechanisms related to beta cell development (56–58) to function (59, 60) and degeneration (61–63), as reviewed recently (64). In the case of novel CHI genes, several parameters can be used to unravel disease mechanisms. These include, at least, development of the endocrine populations during differentiation; insulin secretion under different stimuli *in vitro*; transcriptomic, epigenomic proteomic, and dynamic metabolomic studies. The stem cell based approach can provide even the large amounts of tissue required for these analyses.

The Use of CHI SC-Islets as a Model for Chronic Beta-Cell Hyperfunctionality and Glucotoxicity

The beta cell is highly specialized, focusing on the production and secretion of insulin. It is understandable that CHI mutations, which by definition accelerate this process, have profound consequences for the cellular biology of the beta cells. Huopio and colleagues established that, in addition to causing hypoglycemia in infancy (65), dominant K_{ATP}HI mutations predispose to T2DM later in life (66), providing clinical evidence for the detrimental consequences of long-term beta cell hyperactivity. Similar T2DM predisposing effect has been discovered in carriers of activating glucokinase mutations (GCK-HI) (67, 68).

Li and colleagues found that diverse cellular functions are disturbed or altered in CHI patient beta cells lacking K_{ATP}-channels, including glucose dependent metabolic pathways, expression of key transcription factors and receptors and the regulation of cell cycle (69). Additionally, the chronically elevated $[Ca^{2+}]_i$ characteristic of K_{ATP}HI and GCK-HI compromises beta cell identity in (70) and causes double strand breaks and p53 activation (71). Related to these findings, the increased workload of CHI beta cells initially increases their proliferation and mass while later leading to beta cell dysfunction and apoptosis *via* glucotoxicity (71–74), thus paralleling the natural course of beta cells in type 2 diabetes. These examples highlight the possibility of using CHI SC-islets as a model to discover further consequences of the influence of chronically altered beta cell functional states on their biology. Indeed, we could capture the initially increased beta cell mass and proliferation *in vitro*, while the proliferation normalized *in vivo* (37). Again, the possibility for isogenic comparison between healthy and CHI SC-islets provides a specific means to link any alterations to specific mutations.

CONCLUSIONS

Stem cell derived islets represent a powerful tool for modeling diseases of the pancreatic beta cell, due to the potential to produce them in limitless quantities with high consistency and with high disease phenotype fidelity. In the case of CHI, the SC-islets can be harnessed to discover novel antihypoglycemic medications, to study molecular mechanisms of newly discovered CHI genes and to study the basic biology of a hyperactive beta cell. Thus, we believe that modeling of CHI with SC-islets will serve as a critical next step required for the development of specific and efficient antihypoglycemic drugs.

AUTHOR CONTRIBUTIONS

VL wrote the initial draft and edited the manuscript. TO edited the manuscript and provided resources. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Güemes M, Rahman SA, Kapoor RR, Flanagan S, Houghton JAL, Misra S, et al. Hyperinsulinemic Hypoglycemia in Children and Adolescents: Recent Advances in Understanding of Pathophysiology and Management. *Rev Endocr Metab Disord* (2020) 21(4):577–97. doi: 10.1007/s11154-020-09548-7
2. Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K. Clinical and Molecular Characterisation of 300 Patients With Congenital Hyperinsulinism. *Eur J Endocrinol* (2013) 168(4):557–645. doi: 10.1530/EJE-12-0673
3. Männistö JME, Maria M, Raivo J, Kuulasmaa T, Otonkoski T, Huopio H, et al. Clinical and Genetic Characterization of 153 Patients With Persistent or Transient Congenital Hyperinsulinism. *J Clin Endocrinol Metab* (2020) 105 (4):E1686–945. doi: 10.1210/clinem/dgz271
4. Rosenfeld E, Ganguly A, de Leon DD. Congenital Hyperinsulinism Disorders: Genetic and Clinical Characteristics. *Am J Med Genet Part C: Semin Med Genet* (2019) 181(4):682–925. doi: 10.1002/ajmg.c.31737
5. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, et al. Genotype and Phenotype Correlations in 417 Children With Congenital Hyperinsulinism. *J Clin Endocrinol Metab* (2013) 98(2):355–63. doi: 10.1210/jc.2012-2169
6. Muukkonen L, Männistö J, Jääskeläinen J, Hannonen R, Huopio H. The Effect of Hypoglycaemia on Neurocognitive Outcome in Children and Adolescents With Transient or Persistent Congenital Hyperinsulinism. *Dev Med Child Neurol* (2019) 61(4):451–575. doi: 10.1111/dmcn.14039
7. Roeper M, Dafsari RS, Hoermann H, Mayatepek E, Kummer S, Meissner T. Risk Factors for Adverse Neurodevelopment in Transient or Persistent Congenital Hyperinsulinism. *Front Endocrinol* (2020) 11:580642. doi: 10.3389/fendo.2020.580642
8. Shah R, Harding J, Brown J, Mckinlay C. Neonatal Glycaemia and Neurodevelopmental Outcomes: A Systematic Review and Meta-Analysis. *Neonatology* (2019) 115(2):116–265. doi: 10.1159/000492859
9. Lord K, Radcliffe J, Gallagher PR, Adzick NS, Stanley CA, de León DD. High Risk of Diabetes and Neurobehavioral Deficits in Individuals With Surgically

Treated Hyperinsulinism. *J Clin Endocrinol Metab* (2015) 100(11):4133–395. doi: 10.1210/jc.2015-2539
10. Helleskov A, Melikyan M, Globa E, Shcherderkina I, Poertner F, Larsen AM, et al. Both Low Blood Glucose and Insufficient Treatment Confer Risk of Neurodevelopmental Impairment in Congenital Hyperinsulinism: A Multinational Cohort Study. *Front Endocrinol* (2017) 8:156. doi: 10.3389/fendo.2017.00156
11. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren P-O, Caicedo A. The Unique Cytoarchitecture of Human Pancreatic Islets Has Implications for Islet Cell Function. *Proc Natl Acad Sci USA* (2006) 103(7):2334–95. doi: 10.1073/pnas.0510790103
12. Nair G, Hebrok M. Islet Formation in Mice and Men: Lessons for the Generation of Functional Insulin-Producing β-Cells From Human Pluripotent Stem Cells. *Curr Opin Genet Dev* (2015) 32:171–80. doi: 10.1016/j.gde.2015.03.004
13. Balboa D, Otonkoski T. Human Pluripotent Stem Cell Based Islet Models for Diabetes Research. *Best Pract Res: Clin Endocrinol Metab* (2015) 29(6):899–9095. doi: 10.1016/j.beem.2015.10.012
14. Rodriguez-Diaz R, Molano RD, Weitz JR, Abdulreda MH, Berman DM, Leibiger B, et al. Paracrine Interactions Within the Pancreatic Islet Determine the Glycemic Set Point. *Cell Metab* (2018) 27(3):549–58.e4. doi: 10.1016/j.cmet.2018.01.015
15. Seghers V, Nakazaki M, DeMayo F, Aguilar-Bryan L, Bryan J. Sur1 Knockout Mice. A Model for K(ATP) Channel-Independent Regulation of Insulin Secretion. *J Biol Chem* (2000) 275(13):9270–7. doi: 10.1074/jbc.275.13.9270
16. Miki T, Nagashima K, Tashiro F, Kotake K, Yoshitomi H, Tamamoto A, et al. Defective Insulin Secretion and Enhanced Insulin Action in K ATP Channel-Deficient Mice. *Biochemistry* (1998) 95:10402–6. doi: 10.1073/pnas.95.18.10402
17. Shiota C, Larsson O, Shelton K, Shiota M, Efanov A, Hoy M, et al. Sulfonylurea Receptor Type 1 Knock-Out Mice Have Intact Feeding-Stimulated Insulin Secretion Despite Marked Impairment in Their Response to Glucose. *J Biol Chem* (2002) 277:37176–83. doi: 10.1074/jbc.M206757200
18. Thomson JA. Embryonic Stem Cell Lines Derived From Human Blastocysts. *Science* (1998) 282(5391):1145–7. doi: 10.1126/science.282.5391.1145
19. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, et al. Induction of Pluripotent Stem Cells From Adult Human Fibroblasts by Defined Factors. *Cell* (2007) 131(5):861–72. doi: 10.1016/j.cell.2007.11.019
20. Anzalone AV, Koblan LW, Liu DR. Genome Editing With CRISPR–Cas Nucleases, Base Editors, Transposases and Prime Editors. *Nat Biotechnol* (2020) 38(7):824–44. doi: 10.1038/s41587-020-0561-9
21. D'Amour KA, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, et al. Production of Pancreatic Hormone-Expressing Endocrine Cells From Human Embryonic Stem Cells. *Nat Biotechnol* (2006) 24(11):1392–401. doi: 10.1038/nbt1259
22. Rezania A, Bruin JE, Arora P, Rubin A, Batushansky I, Asadi A, et al. Reversal of Diabetes With Insulin-Producing Cells Derived in Vitro From Human Pluripotent Stem Cells. *Nat Biotechnol* (2014) 32(11):1121–33. doi: 10.1038/nbt.3033
23. Pagliuca FW, Millman JR, Gürtler M, Segel M, Van Dervort A, Ryu JH, et al. Generation of Functional Human Pancreatic β Cells. *In Vitro Cell* (2014) 159 (2):428–39. doi: 10.1016/j.cell.2014.09.040
24. Nostro MC, Sarangi F, Yang C, Holland A, Elefanty AG, Stanley EG, et al. Efficient Generation of NKX6-1+ Pancreatic Progenitors From Multiple Human Pluripotent Stem Cell Lines. *Stem Cell Rep* (2015) 4(4):591–6045. doi: 10.1016/j.stemcr.2015.02.017
25. Russ HA, Parent AV, Ringler JJ, Hennings TG, Nair GG, Shveygert M, et al. Controlled Induction of Human Pancreatic Progenitors Produces Functional Beta-Like Cells *In Vitro*. *EMBO J* (2015) 34(13):e201591058. doi: 10.15252/embj.201591058
26. Toyoda T, Kimura A, Tanaka H, Ameku T, Mima A, Hirose Y, et al. Rho-Associated Kinases and Non-Muscle Myosin IIs Inhibit the Differentiation of Human IPSCs to Pancreatic Endoderm. *Stem Cell Rep* (2017) 9(2):419–28. doi: 10.1016/j.stemcr.2017.07.005
27. Velazco-Cruz L, Song J, Maxwell KG, Goedegebuure MM, Augsornworawat P, Hogrebe NJ, et al. Acquisition of Dynamic Function in Human Stem Cell-Derived Beta Cells. *Stem Cell Rep* (2019) 12(2):351–65. doi: 10.1016/j.stemcr.2018.12.012
28. Hogrebe NJ, Augsornworawat P, Maxwell KG, Velazco-Cruz L, Millman JR. Targeting the Cytoskeleton to Direct Pancreatic Differentiation of Human Pluripotent Stem Cells. *Nat Biotechnol* (2020) 38(4):460–70. doi: 10.1038/s41587-020-0430-6
29. Nair GG, Liu JS, Russ HA, Tran S, Saxton MS, Chen R, et al. Recapitulating Endocrine Cell Clustering in Culture Promotes Maturation of Human Stem-Cell-Derived β Cells. *Nat Cell Biol* (2019) 21(2):263–74. doi: 10.1038/s41556-018-0271-4
30. Balboa D, Barsby T, Lithovius V, Saarimäki-vire J, Omar-hmeadi M, Dyachok O, et al. Functional, Metabolic and Transcriptional Maturation of Human Pancreatic Islets Derived From Stem Cell. *BioRxiv* (2021) 447439:1–20. doi: 10.1038/s41587-022-01219-z
31. Yoshihara E, Connor CO, Gasser E, Wei Z, Oh TG, Tseng TW, et al. Immune-Evasive Human Islet-Like Organoids Ameliorate Diabetes. *Nature* (2020) 586:606–11. doi: 10.1038/s41586-020-2631-z
32. Helman A, Cangelosi AL, Davis JC, Straubhaar JR, Sabatini DM, Melton DA, et al. Article A Nutrient-Sensing Transition at Birth Triggers Glucose-Responsive Insulin Secretion Ll Article A Nutrient-Sensing Transition at Birth Triggers Glucose-Responsive Insulin Secretion. *Cell Metab* (2020) 31 (5):1004–16.e5. doi: 10.1016/j.cmet.2020.04.004
33. Davis JC, Alves TC, Helman A, Liu DR, Kibbey RG, Melton DA, et al. Article Glucose Response by Stem Cell-Derived B Cells In Vitro Is Inhibited by a Bottleneck in Glycolysis Ll Ll Glucose Response by Stem Cell-Derived B Cells *In Vitro* Is Inhibited by a Bottleneck in Glycolysis. *CellReports* (2020) 31 (6):107623. doi: 10.1016/j.celrep.2020.107623
34. Alvarez-Dominguez JR, Donaghey J, Rasouli N, Kenty JHR, Helman A, Charlton J, et al. Circadian Entrainment Triggers Maturation of Human. *In Vitro Islets Cell Stem Cell* (2020) 26(1):108–22.e10. doi: 10.1016/j.stem.2019.11.011
35. Mahaddalkar PU, Scheibner K, Pfluger S, Ansarullah MS, Beckenbauer J, Irmler M, et al. Generation of Pancreatic β Cells From CD177+ Anterior Definitive Endoderm. *Nat Biotechnol* (2020) 38(9):1061–72. doi: 10.1038/s41587-020-0492-5
36. Guo D, Liu H, Ru A, Gao GE, Nasir A. Modeling Congenital Hyperinsulinism With ABCC8 - Deficient Human Embryonic Stem Cells Generated by CRISPR / Cas9. *Sci Rep* (2017) 7(3156):1–8. doi: 10.1038/s41598-017-03349-w
37. Lithovius V, Saarimäki-Vire J, Balboa D, Ibrahim H, Montaser H, Barsby T, et al. SUR1-Mutant iPS Cell-Derived Islets Recapitulate the Pathophysiology of Congenital Hyperinsulinism. *Diabetologia* (2021) 64(3):630–40. doi: 10.1007/s00125-020-05346-7
38. Otonkoski T, Ämmälä C, Huopio H, Cote GJ, Chapman J, Cosgrove K, et al. A Point Mutation Inactivating the Sulfonylurea Receptor Causes the Severe Form of Persistent Hyperinsulinemic Hypoglycemia of Infancy in Finland. *Diabetes* (1999) 48:408–15. doi: 10.2337/diabetes.48.2.408
39. Kellaway SG, Mosinska K, Mohamed Z, Ryan A, Richardson S, Newbould M, et al. Increased Proliferation and Altered Cell Cycle Regulation in Pancreatic Stem Cells Derived From Patients With Congenital Hyperinsulinism. (2019) *PLoS ONE* 14(9):e0222350. doi: 10.1371/journal.pone.0222350
40. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ. Therapies and Outcomes of Congenital Hyperinsulinism-Induced Hypoglycaemia. *Diabetic Med* (2019) 36(1):9–21. doi: 10.1111/dme.13823
41. Henquin JC, Nenquin M, Sempoux C, Guiot Y, Bellanné-Chantelot C, Otonkoski T, et al. In Vitro Insulin Secretion by Pancreatic Tissue From Infants With Diazoxide-Resistant Congenital Hyperinsulinism Deviates From Model Predictions. *J Clin Invest* (2011) 121(10):3932–42. doi: 10.1172/JCI58400
42. Adzick NS, de Leon DD, States LJ, Lord K, Bhatti TR, Becker SA, et al. Surgical Treatment of Congenital Hyperinsulinism: Results From 500 Pancreatectomies in Neonates and Children. *J Pediatr Surg* (2019) 54(1):27–32. doi: 10.1016/j.jpedsurg.2018.10.030
43. Beltrand J, Caquard M, Arnoux J-B, Laborde K, Velho G, Verkarre V, et al. Glucose Metabolism in 105 Children and Adolescents After Pancreatectomy for Congenital Hyperinsulinism. *Diabetes Care* (2012) 35(2):198–203. doi: 10.2337/dc11-1296

44. Arya VB, Senniappan S, Demirbilek H, Alam S, Flanagan SE, Ellard S, et al. Pancreatic Endocrine and Exocrine Function in Children Following Near-Total Pancreatectomy for Diffuse Congenital Hyperinsulinism. *PloS One* (2014) 9(5):4–9. doi: 10.1371/journal.pone.0098054
45. Thomas PM, Cote GJ, Wohilk N, Haddad B, Mathew PM, Rabl W, et al. Mutations in the Sulfonylurea Receptor Gene in Familial Persistent Hyperinsulinemic Hypoglycemia of Infancy. *Science* (1995) 268:426–29. doi: 10.1126/science.7716548
46. Güemes M, Shah P, Silvera S, Morgan K, Gilbert C, Hinchey L, et al. Assessment of Nifedipine Therapy in Hyperinsulinemic Hypoglycemia Due to Mutations in the Abcc8 Gene. *J Clin Endocrinol Metab* (2017) 102(3):822–30. doi: 10.1210/jc.2016-2916
47. Sikimic J, Hoffmeister T, Gresch A, Kaiser J, Barthlen W, Wolke C, et al. Possible New Strategies for the Treatment of Congenital Hyperinsulinism. *Front Endocrinol* (2020) 11:545638. doi: 10.3389/fendo.2020.545638
48. Regard JB, Sato IT, Coughlin SR. Anatomical Profiling of G Protein-Coupled Receptor Expression. *Cell* (2008) 135(3):561–71. doi: 10.1016/j.cell.2008.08.040
49. Amisten S, Salehi A, Rorsman P, Jones PM, Persaud SJ. An Atlas and Functional Analysis of G-Protein Coupled Receptors in Human Islets of Langerhans. *Pharmacol Ther* (2013) 139(3):359–91. doi: 10.1016/j.pharmthera.2013.05.004
50. Gromada J, Bokvist K, Ding WG, Holst JJ, Nielsen JH, Rorsman P. Glucagon-Like Peptide 1(7-36) Amide Stimulates Exocytosis in Human Pancreatic β-Cells by Both Proximal and Distal Regulatory Steps in Stimulus-Secretion Coupling. *Diabetes* (1998) 47(1):57–65. doi: 10.2337/diab.47.1.57
51. de León DD, Li C, Delson MI, Matschinsky FM, Stanley CA, Stoffers DA. Exendin-(9-39) Corrects Fasting Hypoglycemia in SUR-1-/- Mice by Lowering Camp in Pancreatic β-Cells and Inhibiting Insulin Secretion. *J Biol Chem* (2008) 283(38):25786–935. doi: 10.1074/jbc.M804372200
52. Calabria AC, Li C, Gallagher PR, Stanley CA, de León DD. GLP-1 Receptor Antagonist Exendin-(9-39) Elevates Fasting Blood Glucose Levels in Congenital Hyperinsulinism Owing to Inactivating Mutations in the ATP-Sensitive K+ Channel. *Diabetes* (2012) 61(10):2585–91. doi: 10.2337/db12-0166
53. Senniappan S, Alexandrescu S, Tatevian N, Shah P, Arya V, Flanagan S, et al. Sirolimus Therapy in Infants With Severe Hyperinsulinemic Hypoglycemia. *N Engl J Med* (2014) 370(12):1131–37. doi: 10.1056/NEJMoa1310967
54. Szymanowski M, Estebanez MS, Padidela R, Han B, Mosinska K, Stevens A, et al. MTOR Inhibitors for the Treatment of Severe Congenital Hyperinsulinism: Perspectives on Limited Therapeutic Success. *J Clin Endocrinol Metab* (2016) 101(12):4719–29. doi: 10.1210/jc.2016-2711
55. Güemes M, Dastamani A, Ashworth M, Morgan K, Ellard S, Flanagan SE, et al. Sirolimus: Efficacy and Complications in Children With Hyperinsulinemic Hypoglycemia: A 5-Year Follow-Up Study. *J Endocr Soc* (2019) 3(4):699–713. doi: 10.1210/js.2018-00417
56. Saarimäki-Vire J, Balboa D, Russell MA, Saarikettu J, Kinnunen M, Keskitalo S, et al. An Activating STAT3 Mutation Causes Neonatal Diabetes Through Premature Induction of Pancreatic Differentiation. *Cell Rep* (2017) 19(2):281–94. doi: 10.1016/j.celrep.2017.03.055
57. Teo AKK, Lau HH, Valdez IA, Dirice E, Tjora E, Raeder H, et al. Early Developmental Perturbations in a Human Stem Cell Model of MODY5/HNF1B Pancreatic Hypoplasia. *Stem Cell Rep* (2016) 6(3):357–67. doi: 10.1016/j.stemcr.2016.01.007
58. Vethe H, Ghila L, Berle M, Hoareau L, Haaland Ø, Scholz H, et al. The Effect of Wnt Pathway Modulators on Human IPSC-Derived Pancreatic Beta Cell Maturation. *Front Endocrinol* (2019) 10:293. doi: 10.3389/fendo.2019.00293
59. Balboa D, Saarimäki-Vire J, Borshagovski D, Survila M, Lindholm P, Galli E, et al. Insulin Mutations Impair Beta-Cell Development in a Patient-Derived iPSC Model of Neonatal Diabetes. *ELife* (2018) 7:e38519. doi: 10.7554/eLife.38519
60. Zeng H, Guo M, Zhou T, Tan L, Chong CN, Zhang T, et al. An Isogenic Human ESC Platform for Functional Evaluation of Genome-Wide-Association-Study-Identified Diabetes Genes and Drug Discovery. *Cell Stem Cell* (2016) 19(3):326–40. doi: 10.1016/j.stem.2016.07.002
61. de Franco E, Lytrivi M, Ibrahim H, Montaser H, Wakeling MN, Fantuzzi F, et al. YIPF5 Mutations Cause Neonatal Diabetes and Microcephaly Through Endoplasmic Reticulum Stress. *J Clin Invest* (2020) 130(12):6338–53. doi: 10.1172/JCI141455
62. Montaser H, Patel KA, Balboa D, Ibrahim H, Lithovius V, Näätänen A, et al. Loss of MANF Causes Childhood-Onset Syndromic Diabetes Due to Increased Endoplasmic Reticulum Stress. *Diabetes* (2021) 70(4):1006–18. doi: 10.2337/db20-1174
63. Maxwell KG, Augsornworawat P, Velazco-Cruz L, Kim MH, Asada R, Hogrebe NJ, et al. Gene-Edited Human Stem Cell–Derived β Cells From a Patient With Monogenic Diabetes Reverse Preexisting Diabetes in Mice. *Sci Trans Med* (2020) 12(540):eaax9106. doi: 10.1126/scitranslmed.aax9106
64. Balboa D, Iworima DG, Kieffer TJ. Human Pluripotent Stem Cells to Model Islet Defects in Diabetes. *Front Endocrinol (Lausanne)* (2021) 12:1–19. doi: 10.3389/fendo.2021.642152
65. Huopio H, Reimann F, Ashfield R, Komulainen J, Lenko H-l, Rahier J, et al. Dominantly Inherited Hyperinsulinism Caused by a Mutation in the Sulfonylurea Receptor Type 1. *J Clin Invest* (2000) 106: (7):897–906. doi: 10.1172/JCI9804
66. Huopio H, Otonkoski T, Vauhkonen I, Reimann F, Ashcroft FM, Laakso M. A New Subtype of Autosomal Dominant Diabetes Attributable to a Mutation in the Gene for Sulfonylurea Receptor 1. *Lancet* (2003) 361(9354):301–7. doi: 10.1016/S0140-6736(03)12325-2
67. Langer S, Waterstradt R, Hillebrand G, Santer R, Baltrusch S. The Novel GCK Variant P.Val455Leu Associated With Hyperinsulinism Is Susceptible to Allosteric Activation and Is Conducive to Weight Gain and the Development of Diabetes. *Diabetologia* (2021) 64(12):2687–700. doi: 10.1007/s00125-021-05553-w
68. Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, et al. Familial Hyperinsulinism Caused by an Activating Glucokinase Mutation. *N Engl J Med* (1998) 338(4):226–30. doi: 10.1056/NEJM199801223380404
69. Li C, Ackermann AM, Boodhansingh KE, Bhatti TR, Liu C, Schug J, et al. Functional and Metabolomic Consequences of ATP-Dependent Potassium Channel Inactivation in Human Islets. *Diabetes* (2017) 66:db170029. doi: 10.2337/db17-0029
70. Stancill JS, Cartailler JP, Clayton HW, O'Connor JT, Dickerson MT, Dadi PK, et al. Chronic β-Cell Depolarization Impairs β-Cell Identity by Disrupting a Network of Ca2+-Regulated Genes. *Diabetes* (2017) 66(8):2175–87. doi: 10.2337/db16-1355
71. Tornovsky-Babeay S, Dadon D, Ziv O, Tzipilevich E, Kadosh T, Haroush RS-B, et al. Type 2 Diabetes and Congenital Hyperinsulinism Cause DNA Double-Strand Breaks and P53 Activity in β Cells. *Cell Metab* (2014) 19 (1):109–21. doi: 10.1016/j.cmet.2013.11.007
72. Tornovsky-Babeay S, Weinberg-Corem N, Schyr RB-H, Avrahami D, Lavi J, Feleke E, et al. Biphasic Dynamics of Beta Cell Mass in a Mouse Model of Congenital Hyperinsulinism: Implications for Type 2 Diabetes. *Diabetologia* (2021) 64(5):1133–43. doi: 10.1007/s00125-021-05390-x
73. Kassem S, Bhandari S, Rodríguez-Bada P, Motaghedi R, Heyman M, García-Gimeno MA, et al. Large Islets, Beta-Cell Proliferation, and a Glucokinase Mutation. *N Engl J Med* (2010) 362(14):1348–50. doi: 10.1056/nejmc0909845
74. Kassem SA, Ariel I, Thornton PS, Scheimberg I, Glaser B. Beta-Cell Proliferation and Apoptosis in the Developing Normal Human Pancreas and in Hyperinsulinism of Infancy. *Diabetes* (2000) 49(8):1325–33. doi: 10.2337/diabetes.49.8.1325

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REVIEW
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Rare Disease Registries Are Key to Evidence-Based Personalized Medicine: Highlighting the European Experience

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Rare diseases, such as inherited metabolic diseases, have been identified as a health priority within the European Union more than 20 years ago and have become an integral part of EU health programs and European Reference Networks. Having the potential to pool data, to achieve sufficient sample size, to overcome the knowledge gap on rare diseases and to foster epidemiological and clinical research, patient registries are recognized as key instruments to evidence-based medicine for individuals with rare diseases. Patient registries can be used for multiple purposes, such as (1) describing the natural history and phenotypic diversity of rare diseases, (2) improving case definition and indication to treat, (3) identifying strategies for risk stratification and early prediction of disease severity (4), evaluating the impact of preventive, diagnostic, and therapeutic strategies on individual health, health economics, and the society, and (5) informing guideline development and policy makers. In contrast to clinical trials, patient registries aim to gather real-world evidence and to achieve generalizable results based on patient cohorts with a broad phenotypic spectrum. In order to develop a consistent and sustained framework for rare disease registries, uniform core principles have been formulated and have been formalized through the European Rare Disease Registration Infrastructure. Adherence to these core principles and compliance with the European general data protection regulations ensures that data collected and stored in patient registries can be exchanged and pooled in a protected environment. To illustrate the benefits and limitations of patient registries on rare disease research this review focuses on inherited metabolic diseases.

Keywords: rare disease (RD), inherited metabolic diseases, FAIR (findable, accessible, interoperable, and reusable) principles, personalized medicine, patient registry

INTRODUCTION

Any disease affecting less than 1 in 2,000 people is considered rare in the European Union (EU). Although the approximately 6,000-8,000 distinct rare diseases are highly heterogeneous regarding etiology, pathophysiology, clinical presentation, and treatability, they have in common that affected individuals are often confronted with similar problems, such as diagnostic odyssey, lack of safe and effective therapies, and scarce specialist centers, resulting in significant inequalities. On the European level, rare diseases were identified by the 2nd EU Health Program as a healthcare priority with unmet needs, highlighting patient registries as the best way to pool data in order to achieve sufficient sample sizes for epidemiological and clinical research (1).

Rare diseases such as inherited metabolic diseases have been traditionally regarded as Mendelian traits that are caused by specific pathogenic gene variations, and as experiments of nature which helped to decipher underlying mechanisms of monogenic disorders (2). Natural history studies on inherited metabolic diseases, however, often failed to identify clear-cut genotype-phenotype correlations and to predict the clinical phenotype based on the genotype or biochemical phenotype. This is partially explained by involvement of other pathways whose fluxes are changed though secondary inhibition induced by toxic metabolites or lack of substrates (3, 4). Furthermore, modifier genes (5, 6), alternative pathways (7–9), side reactions of enzymes (10), metabolic proof-reading (11), and the concomitant net effect of metabolic changes on the level of single cells, organs and the whole organism significantly contribute to the resulting phenotype. Besides these intrinsic factors, it has been increasingly recognized that metabolism can also be influenced by gene-environment and gene-nutrient interactions (12–14), and the microbiome (15, 16). As a consequence, inherited metabolic diseases are nowadays perceived as a model for complex diseases. Another important aspect that causes apparent phenotypic diversity is time, the fourth dimension setting the scene for ontogeny and ageing. Therefore, the phenotype of an individual at a certain time point of life should not be regarded as static but as a snapshot. Any phenotypic comparisons should include the individual age as an anchor point, the clinical phenotype itself being a complex moving target.

For rare diseases patient registries are thought to be key instruments to achieve a sufficient sample size for clinical research, to guide healthcare planning, and to foster the development and evaluation of diagnostic and therapeutic interventions. Patient registries significantly contribute to evidence-based personalized medicine in the field of rare diseases, since they can be used for multiple purposes, such as improvement of case definition, revision of disease classification, evaluation of indication to treat and risk stratification, as well as safety, effectiveness, feasibility, limitations and benefits of diagnostic and therapeutic strategies under real-world conditions.

THE EVOLUTION OF RARE DISEASE REGISTRIES

From Single Diseases to European Reference Networks (ERN) Registries

With the benefits of patient registries being widely accepted, the field of rare diseases saw a multitude of individual efforts on registry building in the past, usually realized within a national framework and focusing on single diseases or tightly defined disease groups. Successful examples on a national level include the mitoREGISTRY of the German Network for Mitochondrial Disorders (mitoNET, https://www.mitonet.org/) as well as Leukonet (https://www.leukodystrophie-info.de/), the German Network for Leukodystrophies, gathering data on mitochondrial disease since 2009 and leukodystrophies since 2003 respectively, both receiving funding from the German Federal Ministry of Education and Research (BMBF). International examples are the EUROGLYCANET, targeting congenital disorders of glycosylation (CDG) since 1999 (17) and the European Rare Kidney Disease Registry (ERKReg), observing rare kidney disease since 2018 (18) both having received funding from the European Commission, or the Urea Cycle Disorders Consortium (UCDC), having received funding from the US-American National Institutes of Health (NIH) since 2003 (19). While these are notable examples, they of course represent only a small sample of the wealth of existing registries, which currently are estimated as 793 only within the scope of Orphanet (20). Therefore the landscape of rare disease registries was highly scattered when EU policy makers recognized as early as 1999, the urgency of action on rare diseases at the European level (21), starting a process that lead to the recognition of scientific patient registries as key instruments to overcome challenges specific to rare diseases, like small sample sizes, while also envisioning the introduction of European Reference Networks as a means of organizing centers of excellence (22). This resulted in rare diseases becoming an integral part of the second EU health programme, calling for funding opportunities for the development and maintenance of rare disease registries (23). In the field of inherited metabolic diseases this development allowed the founding of the European Registry and Network for Intoxication type Metabolic Diseases (E-IMD: https://www.eimd-registry.org/; CHAFEA agreement no. 2010 12 01) in 2010, the European Network and Registry for Homocystinurias and Methylation Defects (E-HOD: https://www.ehod-registry.org/; CHAFEA agreement no. 2012 12 02) in 2012, followed by the International Working Group on Neurotransmitter-Related Disorders (iNTD: https://www.intd-registry.org/) in 2014, as expert lead international patient registries (24–26). Due to the increasing number of rare inherited metabolic diseases (currently more than 1,600 diseases according to IEMbase, URL: http://www.iembase.org/), these registries were conceptualized as multi-disease registries, logically grouping diseases by common traits of clinical manifestation or affected metabolic pathways. The registries rely on a tailored custom-made IT solution, allowing secure remote entry and central storage of pseudonymized data, while enrolling

patients on the protocol of an observational study, adapted to the local legal context of participating health care providers (see **Figure 1** which illustrates the modular design of electronic case report forms and study visits). The enrolment follows a top-down approach, i.e. large expert centers with great outreach in their specific countries and regions are motivated *via* their inherent scientific interest to join the consortia and enroll known and newly diagnosed patients on the various protocols. This process ensures an overall high quality of data through the expert-mediated enrolment and data entry process, leading to a good coverage for diseases whose management necessitates regular visits to large expert centers. On the other hand, this process could result in under-reporting where a more bottom-up approach mediated by patient organizations might be more beneficial. The associated health care providers organized themselves in scientific consortia, granting each member the right to use the entire wealth of gathered data for sufficiently elaborated projects, presented to and agreed by a member's board, while at the same time guaranteeing strict individual ownership of data, thus still allowing single center research or evaluation of national cohorts, even including the right to opt out of common projects, if conflict mitigation mechanisms should fail.

The data model employed by each registry is the result of the collaborative effort of experts as well as patient organizations in the field, being perfectly suitable for capturing clinical phenotypes, but nevertheless meeting more recently established standards of semantic interoperability only to a restricted extent.

European Rare Disease Registration Infrastructure (ERDRI) and Semantic Interoperability

Concomitantly to promoting individual registry building efforts the European Commission also furthered the development of the underlying framework for actions in the field of rare diseases, by establishing the advisory body of the European Union Committee of Experts on Rare Diseases (EUCERD) among others (27). The resulting EUCERD core recommendations on rare disease patient registration and data collection emphasize the need for establishing minimum common data sets, which should be uniform not only among disease specific registries but also across the entire spectrum of rare diseases, stress the critical importance of semantic interoperability between registries, which can be achieved by using standardized and controlled languages like OMIM code for case definition or SNOMED-CT for describing clinical phenotypes and endorse the introduction of a common European patient identifier (28). These principles should later materialize in the form of the European Rare Disease Registration Infrastructure (ERDRI), an integrated set of tools and recommendations presented on the European Platform for Rare Diseases. The central instrument of ERDRI is the Common Data Elements (CDE), a set of defined variables that should form the core of all European rare disease registries along with concrete recommendations on the usage of controlled dictionaries like Orpha or Alpha code for coding diseases, the nomenclature of the Human Genome Variation Society (HGVS) for describing the genetic phenotype and the Human Phenotype Ontology (HPO) for the clinical phenotype. The CDEs are embedded with further tools comprising an European metadata repository (ERDRI.mdr), an European reference database for rare disease registries (ERDRI.dor) and the introduction of an unique European patient identifier (European Platform on Rare Diseases Registration (29). With European Reference Networks having become a reality, by the time the ERDRI tools were released, the Unified Registry for Inherited Metabolic Disorders (U-IMD; CHAFEA agreement no. 777259) could be implemented as the first European and international registry encompassing all inherited metabolic diseases. U-IMD implements the ERDRI and serves as the official registry of the European Reference Network

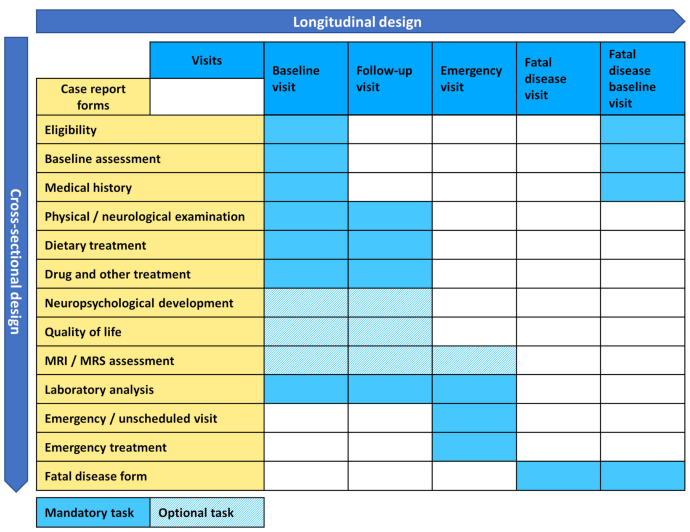

FIGURE 1 | General design of the E-IMD, E-HOD, iNTD and U-IMD registries.

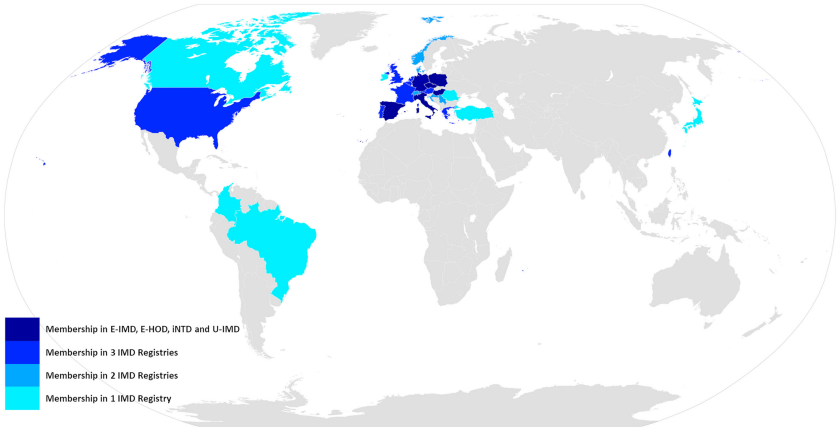

FIGURE 2 | Global outreach of the E-IMD, E-HOD, iNTD and U-IMD registries.

for Hereditary Metabolic Disorders (MetabERN) (see **Figure 2** for an illustration the combined global outreach of the European IMD registries and **Table 1** for the combined current enrolment numbers). Apart from introducing a new registry, the U-IMD action also aims at providing the expertise for implementing a higher level of semantic interoperability to the existing IMD registries. In this way U-IMD works as a complement and enhancement to the existing structures, avoiding duplication much as possible for a generic approach (30). The hurdles represented by lack of semantic interoperability were experienced by E-IMD in its collaborative effort to establish the largest continuously followed cohort of urea cycle disorders together with the American Urea Cycle Disorders Consortium (UCDC). Although surmountable the datasets required extensive work-up by clinicians to correctly classify clinical abnormalities since semantic interoperability of both registries was incomplete (31). Introduction of HPO terms and other controlled and structured dictionaries could have significantly shortened the mapping process (32). With these lessons in mind the adaption process facilitated by U-IMD includes the introduction of the EDRI tools to the E-IMD, E-HOD and iNTD registries, with special emphasis enhancing semantic interoperability between the registry and to other data sources with a comparable standard. U-IMD itself ensured interoperability to other ERN registries already during its design, going as far as mirroring the data model of the European Rare Kidney Disease Reference Network (ERKNet) since both ERNs share metabolic nephropathies as target diseases (30).

PATIENT REGISTRIES ARE MULTI-PURPOSE INSTRUMENTS FOR RARE DISEASE RESEARCH

Natural History Studies

The most common application for patient registries is natural history studies, e.g., ultra-rare diseases such as mevalonic aciduria (33), to raise awareness. The precise description of the initial presentation helps to reduce diagnostic delay and to guide the diagnostic process. Noteworthy, phenotypic diversity can be extreme such as in urea cycle disorders, ranging from life-threatening sepsis-like hyperammonemic encephalopathy in newborns with severe disease onset to variable fluctuating episodes of neurological, gastrointestinal and psychiatric symptoms in patients with attenuated forms (34, 35). Phenotypic diversity of variant disease courses can be easily mistaken as discrepant diseases with overlapping biochemical and clinical presentations. This is exemplified by genotype phenotype correlation studies in neuroblastoma-amplified sequence (NBAS)-associated disease which identified three different but partially overlapping subgroups with complex phenotypes which are directly related to the affected region of the NBAS protein (36). In order to distinguish the initial presentation and the evolving clinical phenotypes of rare diseases, comparative phenotyping within and between disease groups is a helpful strategy. Recently, this approach was successfully applied to elucidate clinical similarities and differences between disorders of biogenic amine and tetrahydrobiopterin (BH_4) metabolism (37), urea cycle disorders, and organic acidurias (24). This knowledge is of relevance for the optimization of the diagnostic process, patient clinical paths, healthcare planning, and counselling of parents having a child with a rare disease. For example, comparative phenotyping helped to identify the need to carefully monitor renal function not only in individuals with isolated methylmalonic acidurias but also in individuals with other organic acidurias such as propionic aciduria and glutaric aciduria type 1 (24, 38) since chronic kidney dysfunction was already well known in methylmalonic aciduria (39, 40), but was underestimated in propionic aciduria and unknown in glutaric aciduria type 1 before. Patient registries also allow comparison between different diagnostic journeys and related health outcomes on an international level, especially if there are known rigid differences in diagnostic approaches between countries, that may be evaluated against each other, as is the case with differing national newborn screening panels. Results from such comparisons may help in harmonizing

TABLE 1 | Enrolment of active patients for the E-IMD, E-HOD, iNTD and U-IMD registries and for the German newborn screening outcome study (NGS2020, DRKS00013329).

Disease Groups according to IEM nosology	E-IMD	E-HOD	iNTD	NGS 2020	U-IMD	Total
Disorders of ammonia detoxification	570			17	124	711
Disorders of branched-chain amino acid metabolism	486				153	639
Disorders of lysine metabolism	275			10	40	325
Disorders of cobalamin metabolism	86	222		5	76	389
Disorders of sulfur amino acid and sulfide metabolism		416			54	470
Disorders of folate metabolism		72	5	1	6	84
Disorders of phenylalanine and tetrahydrobiopterin metabolism			186	169	354	709
Disorders of monoamine metabolism			136		2	138
Disorders of β- and γ-amino acids			45		1	46
Disorders of glycine metabolism			29		5	34
Disorders of serine metabolism			5			5
Disorders of fatty acid oxidation and transport				206	182	388
Disorders of branched-chain amino acid metabolism				73		73
Disorders of galactose metabolism				25	60	85
Disorders of biotin metabolism				20	46	66
Disorders of carnitine metabolism				9	25	34
Disorders of tyrosine metabolism				6	28	34
Disorders of riboflavin metabolism				4	16	20
Sphingolipidoses					208	208
Glycogen storage diseases					117	117
Disorders of cholesterol biosynthesis					72	72
Mucopolysaccharidoses					66	66
Disorders of mitochondrial tRNA					48	48
Disorders of lysosomal cholesterol metabolism					43	43
Disorders of gluconeogenesis					40	40
MITOCHONDRIAL DISORDERS OF ENERGY METABOLISM					109	109
DISORDERS OF CARBOHYDRATES					64	64
DISORDERS OF NITROGEN-CONTAINING COMPOUNDS					58	58
CONGENITAL DISORDERS OF GLYCOSYLATION					39	39
DISORDERS OF PEROXISOMES AND OXALATE					33	33
DISORDERS OF LIPIDS					30	30
STORAGE DISORDERS					21	21
DISORDERS OF VITAMINS, COFACTORS AND MINERALS					11	11
DISORDERS OF TETRAPYRROLES					2	2
Total	**1417**	**710**	**407**	**545**	**2133**	**5212**

All patient numbers as of 30th November 2021.

newborn screening programs by illustrating different needs for long term care resulting from different diagnostic journeys in the light of health economics.

Patient registries are powerful tools to describe and compare disease variants and to challenge existing case definition, disease classification, and indication to treat. Recently, careful biochemical and clinical evaluation of more than 300 individuals with confirmed cystathionine β-synthase deficiency resulted in the development of comprehensive criteria for the classification of pyridoxine responsiveness (41). This reclassification is likely to have enormous practical consequences on the diagnostic process, treatment and management of affected individuals with this disease since pyridoxine responsiveness is the major predictor of clinical severity and outcome. Furthermore, patient registries can help to elucidate the clinical relevance of newly identified disease variants with yet uncertain clinical significance. A good example is so-called mild isovaleric aciduria, an attenuated and putatively benign disease variant, which has been increasingly recognized since the inclusion of isovaleric aciduria into newborn screening panels. In contrast to individuals with the severe, so-called classic, disease variant with high risk of neonatal mortality, poor neurocognitive outcome in surviving individuals, and a clear indication to treat, individuals with the mild disease variant are not confronted with acute metabolic decompensations and increased neonatal mortality and showed excellent neurocognitive outcomes. Noteworthy, excellent health outcomes in individuals with mild isovaleric aciduria did not appear to be impacted by metabolic maintenance therapy (42), questioning the overall benefit from NBS and the indication to treat.

A solid description of the disease course with its age-dependent manifestations and variations is also an important source for the identification of meaningful endpoints for clinical trials instead of surrogate parameters with doubtful clinical relevance for affected individuals. Furthermore, it can guide the inclusion of individuals with well-defined clinical severity to clinical trials in order to avoid apparent (non-) effectiveness of the study drug due to case mix differences of study groups. For individuals with urea cycle disorders, the severity of the initial presentation, represented by age at disease onset, initial peak ammonium concentration in plasma, and coma, has a major impact on survival and neurocognitive outcomes in urea cycle disorders (43, 44). In addition to mortality as a hard clinical end

point, collaborative evaluation of 300 individuals with ornithine transcarbamylase deficiency identified intelligence as another suitable clinical endpoint since it was shown to be the most distinguished indicator for cognitive function and to correlate highly with the initial peak ammonium concentration in plasma (45, 46).

Benefits and Limitations of Current Diagnostic and Therapeutic Strategies

It is a well-known fact that individuals with rare diseases are often confronted with a diagnostic odyssey and diagnostic delay, putting high burden on the affected individual and her/his family and being associated with an increased risk of losing therapeutic effectiveness and developing irreversible organ dysfunction. To shorten the path to diagnosis newborn screening programs for rare diseases, such as inherited metabolic diseases, have been continuously developed for more than 50 years following Robert Guthrie's pioneering work (47). With technological developments, such as tandem mass spectrometry, and concomitant extensions of the disease panel, endogenous intoxication-type metabolic diseases with acute neonatal manifestations have been introduced to these programs (48, 49). As a consequence, some individuals affected by these diseases may become symptomatic in the first days of life, even before newborn screening results are available, questioning the benefit of newborn screening. Comparison of screened and unscreened cohorts followed by patient registries can help to estimate the proportion of individuals who can be reliably identified pre-symptomatically depending on the time point of sample collection (44, 50). Such studies can also evaluate the diagnostic process quality of newborn screening programs, identifying current strengths and weaknesses of its team-players, i.e. senders, mail services, and laboratories, evaluate promising new biomarkers, such as 3-O-methyldopa in dried blood spots for aromatic L-amino-acid decarboxylase deficiency, and thus can highlight the potential for improvement (33, 51). Longitudinal outcome studies of newborn screening cohorts, which provide formal evidence of the clinical effectiveness and the long-term health benefits of screened individuals, have remained the neglected part of newborn screening programs. A long-term observational study in Germany recently demonstrated that newborn screening for inherited metabolic diseases resulted in overall normal development (96%), normal cognitive outcome (mean IQ: 100), and regular attendance of screened individuals at kindergarten (95%) and primary school (95%) (51). At the same time, it identified disease-specific variations of health outcomes and diseases with less favorable health outcomes, particularly if recommended treatment was not adequately prescribed or adhered to (38, 52), as well as individuals with a putatively benign disease variants with risk of over-treatment (42). In addition, observational studies based on patient registries are helpful to evaluate the prevalence and putative health impact of early diagnosis and treatment for conditions that are not yet regularly included in national screening panels, such as neonatal vitamin B_{12} deficiency mostly due to hitherto undiagnosed maternal vitamin B_{12} deficiency (53).

Another important but sometimes difficult application of patient registries is the evaluation of therapeutic safety and effectiveness under real-world conditions. In contrast to clinical trials, inclusion and exclusion criteria are kept to a minimum in patient registries in order to study affected individuals with a broad phenotypic spectrum (1). Furthermore, patients are observed as they present for care instead of asking for strict adherence to protocol. Because of the resulting high variation, which is important to achieve generalizable results, evidence-based answers to therapy-related questions often depend on a large sample size and hence require the pooling of data from different sources (44, 46). Interoperability is key to the success of this effort. If these hurdles can be overcome, patient registries are valuable sources for the optimization of therapies and guideline development. For instance, long-term follow-up of the largest cohort of individuals with urea cycle disorders did not find evidence for superiority of any of the available nitrogen scavenger therapies but highlighted that early liver transplantation appears to be beneficial (46). Another example is glutaric aciduria type 1, which was thought to be an untreatable condition in the pre-screening era since treatment given to symptomatic patients did not improve the clinical outcome (54). Introduction of tandem mass spectrometry-based newborn screening programs challenged this view, proving that pre-symptomatic start of treatment is the major prerequisite for favorable neurological outcome (38, 52). Long-term follow-up of two large patient cohorts in the USA and Germany further elucidated that in screened individuals with glutaric aciduria type 1 therapeutic quality and adherence to evidence-based recommendations become major predictors of neurological outcome and survival (55). Furthermore, these studies showed that low lysine diet supplemented with fortified metabolic formula is superior to protein restriction and allows for normal anthropometric long-term development (38, 56, 57). In another large group of individuals with protein-dependent inherited metabolic disorders, study of the dietary impact on growth highlighted the need for careful monitoring of plasma amino acids, particularly branched-chain amino acids and L-arginine, and to adjust the protein-to-energy prescription of diet to support normal growth (58). In brief, besides precise evaluation of the natural history patient registries are also valuable instruments to assess the iatrogenic impact on the evolving clinical phenotype.

Guideline Development

Patient registries are important data sources for guideline development, helping to improve the evidence level and the grade of recommendation. Since guideline development for rare diseases is often difficult and based on literature with a low level of evidence, such as case reports, small case control or cohort studies, all of them having a high risk of bias and low probability that the reported causes and effects are causally linked. Furthermore, guideline development often identifies topics which have remained white spots on the scientific map, with no published evidence at all. Observational studies and health economic evaluations based on patient registries can specifically address these questions and topics to larger cohorts,

seeking for evidence. Furthermore, patient registries can inform guideline developmental groups about the feasibility, safety, and effectiveness of guideline recommendations, paving the way for successful guideline revision. The high potential of this approach can be illustrated by guideline development for glutaric aciduria type 1, being initiated more than 15 years ago. While the first guideline mostly included grade D and a few grade C recommendations, reflecting significant uncertainty (59), the level of evidence and grade of recommendations have been improved continuously, demonstrated by the first (60) and second revision (61). This progress was much accelerated by careful long-term follow-up of national and international cohorts (38, 56, 57, 59, 61–69), demonstrating that (i) newborn screening is the prerequisite of favorable neurological outcome and survival and (ii) is highly cost-effective, and that (iii) adherence to recommended maintenance and emergency treatment is associated with the most favorable neurological outcome and (iv) normal growth. Furthermore, these studies described a so far unknown renal disease manifestation, unraveled similarities (risk of striatal necrosis with concomitant complex movement disorder with predominant dystonia) and discrepancies (cognitive function, white matter changes, subdural hematoma) between biochemically delineated subgroups (high versus low excreter phenotype), and evaluated which part of the complex clinical spectrum can be specifically targeted and changed by current therapy, highlighting the need for safer and more effective medicines. Similar approaches to continuous improvement of guidelines for rare diseases through long-term observational studies coordinated by international scientific consortia have also been chosen for other rare diseases, such as urea cycle disorders (70, 71), propionic and methylmalonic aciduria (72, 73), cobalamin-related remethylation disorders (74–76), cystathionine beta-synthase deficiency (41, 77), and neurotransmitter-related disorders (37, 78, 79).

Post-Authorization Safety Studies (PASS)

Large scale data collection whether in clinical research or other scientific fields, raises concerns regarding the principles of long-term data stewardship, having resulted in the formulation of the FAIR concept, signifying findability, accessibility, interoperability and reusability of data (80). The aspect of reutilization has a special ethical significance in clinical research for rare disease, since comparatively small patient cohorts imply an enhanced risk of putting the burden of participation in multiple studies on a small number of patients, often severely affected. For the same reason of small patient numbers, orphan drug designations and the resulting need for post-authorization safety studies (PASS) are common for rare diseases, which usually are implemented as industry-driven single purpose drug registries. There is an increasing awareness of regulating bodies like the European Medicine Agency (EMA) for the under-utilization of existing data sources like scientific disease registries, which often already gather the type of longitudinal data also required for certain regulatory actions like post-authorization measures (81, 82). Therefore, the collaboration between marketing authorization holders (MAH) and scientific patient registries, within the model of a public private partnership, is a feasible approach for achieving the goals of certain drug safety measures, while at the same time reducing data fragmentation and duplication and ensuring ongoing data stewardship by publicly funded scientific consortia, better capable and more committed to ensuring data FAIR-ness than industry lead efforts. In the past E-HOD, as well as currently E-IMD, successfully implemented the model as envisioned by the EMA, for the orphan drugs Cystadane® (betaine anhydrous, http://www.encepp.eu/encepp/viewResource.htm?id=40022) and Ravicti® (glycerol phenylbutyrate, EUPAS17267; URL: http://www.encepp.eu/encepp/viewResource.htm?id=30377), respectively. In both cases the PASS studies were implemented on a protocol drafted by the MAH together with the scientific registries and accepted by the Pharmacovigilance Risk Assessment Committee (PRAC) of the EMA. Patients already being enrolled in the respective registries could choose to also participate in the PASS, allowing their already available, purely observational data to be used for facilitating the surveillance measure, without increasing their burden or generating new data that would be only available in an industry owned data source.

DISCUSSION

Patient registries are key to personalized evidence-based medicine for individuals with rare diseases since they help to overcome the intrinsic obstacles of rare disease research through pooling of data and achievement of sufficient sample sizes. There is an increasing number of examples demonstrating that patient registries can fulfil multiple purposes for rare disease research, particularly (A) improving the knowledge about natural history and variant disease courses, (B) identifying meaningful endpoints for clinical trials, improving case definition, genotype phenotype correlation, risk stratification, and therapeutic decision, (C) evaluating the safety, effectiveness, and long-term health benefits as well as the health economical and societal benefits of preventive care programs, diagnostic strategies, and therapies, such as orphan drugs, and (D) increasing the evidence base and recommendation grade of guidelines.

However, successful establishment, sustainability, and usefulness of patient registries may be limited by (A) data fragmentation and duplication because of uncoordinated parallel activities with unharmonized data models, (B) lack of seeding and sustainability funding though industry-independent public sources, hampering projects *via* numerous funding dependent constraints like reimbursement of person hours invested by experts, (C) insufficient geographical coverage resulting in small cohorts, (D) non-adherence to data FAIRness, particularly syntactic and semantic interoperability, hampering data exchange and cross-site analysis, (E) non-compliance with data protection regulations resulting in the discontinuation of some older patient registries following the inception of the GDPR in 2018, and (F) non-involvement of patients and patient groups in the development of registries, leading to insufficient consideration of the patients' view and experience. This last point is of special significance, since conservative treatment options for many IMDs can put a high

psychosocial burden on patients and caregivers alike, often necessitating measures like permanent strict dietary management that conflict with many life choices. Adequately capturing these burdens with tailored patient reported outcome measures (PROMs) was often neglected in the past, but will become increasingly important going forward, since novel treatments directly addressing underlying disease causes will increasingly become available, with PROMs being an important measure for their efficacy (83).

In conclusion, the future success of patient registries for rare disease research in Europe critically depends on the compliance with formal requirements, particularly the FAIR data principles and the GDPR to ensure long-term data collection and data exchange in a protected environment. As indicated by a recent survey among 40 rare disease registries, there seems to be a high level of recognition within the community that adherence to formal aspects of governance, particularly FAIR principles, is a key criterion of successful implementation and administration of rare disease registries (84), although especially long-running projects might struggle to keep pace with adapting to recently introduced standards. Particular attention should be paid to facilitating the achievement of ethical approval and informed consent since these hurdles are importing rate-limiting steps (19, 24, 30). With the future development of safe and reliable IT tools for data extraction from electronic health records it is also hoped that the work- and cost-intensive process of gathering and entering personalized health data to registries can be significantly reduced. Finally, industry-independent sustainability of patient registries conducted by scientific consortia and European Reference Networks for Rare Diseases requires the establishment of long-term funding strategies on the level of the European Union and its Member States.

AUTHOR CONTRIBUTIONS

All authors conceptualized the manuscript. SK and FG wrote the first draft of the manuscript. UM and TO edited the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Gliklich RE, Dreyer NA, Leavy MB. AHRQ Methods for Effective Health Care. In: RE Gliklich, NA Dreyer, MB Leavy, editors. *Registries for Evaluating Patient Outcomes: A User's Guide.* Rockville (MD): Agency for Healthcare Research and Quality (Us) (2014). Available at: http://www.effectivehealthcare.ahrq.gov/registries-guide-3.cfm.
2. Lanpher B, Brunetti-Pierri N, Lee B. Inborn Errors of Metabolism: The Flux From Mendelian to Complex Diseases. *Nat Rev Genet* (2006) 7(6):449–60. doi: 10.1038/nrg1880
3. Dimitrov B, Molema F, Williams M, Schmiesing J, Mühlhausen C, Baumgartner MR, et al. Organic Acidurias: Major Gaps, New Challenges, and a Yet Unfulfilled Promise. *J Inherit Metab Dis* (2021) 44(1):9–21. doi: 10.1002/jimd.12254
4. Kölker S, Burgard P, Sauer SW, Okun JG. Current Concepts in Organic Acidurias: Understanding Intra- and Extracerebral Disease Manifestation. *J Inherit Metab Dis* (2013) 36(4):635–44. doi: 10.1007/s10545-013-9600-8
5. Ravaglia S, Malovini A, Cirio S, Danesino C, de Filippi P, Moggio M, et al. Polymorphism in Exercise Genes and Respiratory Function in Late Onset Pompe Disease (LOPD). *J Appl Physiol (Bethesda Md: 1985)* (2021) 131 (6):1762–71. doi: 10.1152/japplphysiol.00154.2020
6. Mecklenburg N, Kowalczyk I, Witte F, Görne J, Laier A, Mamo TM, et al. Identification of Disease-Relevant Modulators of the SHH Pathway in the Developing Brain. *Development* (2021) 148(17):1–17. doi: 10.1242/dev.199307
7. Bezerra GA, Foster WR, Bailey HJ, Hicks KG, Sauer SW, Dimitrov B, et al. Crystal Structure and Interaction Studies of Human DHTKD1 Provide Insight Into a Mitochondrial Megacomplex in Lysine Catabolism. *IUCrJ* (2020) 7(Pt 4):693–706. doi: 10.1107/S205225252000696X
8. Biagosch C, Ediga RD, Hensler SV, Faerberboeck M, Kuehn R, Wurst W, et al. Elevated Glutaric Acid Levels in Dhtkd1-/Gcdh- Double Knockout Mice Challenge Our Current Understanding of Lysine Metabolism. *Biochim Biophys Acta Mol Basis Dis* (2017) 1863(9):2220–8. doi: 10.1016/j.bbadis.2017.05.018
9. Leandro J, Khamrui S, Wang H, Suebsuwong C, Nemeria NS, Huynh K, et al. Inhibition and Crystal Structure of the Human DHTKD1-Thiamin Diphosphate Complex. *ACS Chem Biol* (2020) 15(8):2041–7. doi: 10.1021/acschembio.0c00114
10. Van Schaftingen E, Veiga-da-Cunha M, Linster CL. Enzyme Complexity in Intermediary Metabolism. *J Inherit Metab Dis* (2015) 38(4):721–7. doi: 10.1007/s10545-015-9821-0

11. Veiga-da-Cunha M, Van Schaftingen E, Bommer GT. Inborn Errors of Metabolite Repair. *J Inherit Metab Dis* (2020) 43(1):14–24. doi: 10.1002/jimd.12187
12. Kawase T, Nagasawa M, Ikeda H, Yasuo S, Koga Y, Furuse M. Gut Microbiota of Mice Putatively Modifies Amino Acid Metabolism in the Host Brain. *Br J Nutr* (2017) 117(6):775–83. doi: 10.1017/S0007114517000678
13. Krumsiek J, Mittelstrass K, Do KT, Stückler F, Ried J, Adamski J, et al. Gender-Specific Pathway Differences in the Human Serum Metabolome. *Metabolomics* (2015) 11(6):1815–33. doi: 10.1007/s11306-015-0829-0
14. Shin SY, Fauman EB, Petersen AK, Krumsiek J, Santos R, Huang J, et al. An Atlas of Genetic Influences on Human Blood Metabolites. *Nat Genet* (2014) 46(6):543–50. doi: 10.1038/ng.2982
15. Howard EJ, Lam TKT, Duca FA. The Gut Microbiome: Connecting Diet, Glucose Homeostasis, and Disease. *Annu Rev Med* (2021) 73:469–81. doi: 10.1146/annurev-med-042220-012821
16. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal Microbiota Metabolism of L-Carnitine, a Nutrient in Red Meat, Promotes Atherosclerosis. *Nat Med* (2013) 19(5):576–85. doi: 10.1038/nm.3145
17. Matthijs G. Research Network: EUROGLYCANET: A European Network Focused on Congenital Disorders of Glycosylation. *Eur J Hum Genet* (2005) 13(4):395–7. doi: 10.1038/sj.ejhg.5201359
18. Bassanese G, Wlodkowski T, Servais A, Heidet L, Roccatello D, Emma F, et al. The European Rare Kidney Disease Registry (ERKReg): Objectives, Design and Initial Results. *Orphanet J Rare Dis* (2021) 16(1):251. doi: 10.1186/s13023-021-01872-8
19. Batshaw ML, Tuchman M, Summar M, Seminara J. A Longitudinal Study of Urea Cycle Disorders. *Mol Genet Metab* (2014) 113(1-2):127–30. doi: 10.1016/j.ymgme.2014.08.001
20. Orphanet. *Rare Disease Registries in Europe* (2000). Available at: https://www.orpha.net/orphacom/cahiers/docs/GB/Registries.pdfhttps://www.orpha.net/orphacom/cahiers/docs/GB/Registries.pdf (Accessed November 19, 2021).
21. European Commission. *PROGRAMME OF COMMUNITY ACTION ON RARE DISEASES (1999 – 2003)* (1999). Available at: https://ec.europa.eu/health/archive/ph_overview/previous_programme/rare_diseases/raredis_wpgm99_en.pdfhttps://ec.europa.eu/health/archive/ph_overview/previous_programme/rare_diseases/raredis_wpgm99_en.pdf (Accessed November 11, 2021).
22. European Commission. *Commission Communication Rare Diseases: Europe's Challenge* (2008). Available at: https://ec.europa.eu/health/ph_threats/non_com/docs/rare_com_en.pdfhttps://ec.europa.eu/health/ph_threats/non_com/docs/rare_com_en.pdf (Accessed November 06, 2021).
23. European Commission. *COMMISSION DECISION of 27 February 2008 on the Adoption of the Work Plan for 2008 for the Implementation of the Second Programme of Community Action in the Field of Health (2008-2013), and on the Selection, Award and Other Criteria for Financial Contributions to the Actions of This Programme* (2008). Available at: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ%3AL%3A2008%3A056%3A0036%3A0062%3AEN%3APDFhttps://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ%3AL%3A2008%3A056%3A0036%3A0062%3AEN%3APDF (Accessed November 06, 2021).
24. Kölker S, Dobbelaere D, Häberle J, Burgard P, Gleich F, Summar ML, et al. Networking Across Borders for Individuals With Organic Acidurias and Urea Cycle Disorders: The E-IMD Consortium. *JIMD Rep* (2015) 22:29–38. doi: 10.1007/8904_2015_408
25. Opladen T, Cortès-Saladelafont E, Mastrangelo M, Horvath G, Pons R, Lopez-Laso E, et al. The International Working Group on Neurotransmitter Related Disorders (iNTD): A Worldwide Research Project Focused on Primary and Secondary Neurotransmitter Disorders. *Mol Genet Metab Rep* (2016) 9:61–6. doi: 10.1016/j.ymgmr.2016.09.006
26. European Commission. *EU Health Programme: 2012 Abstracts of the Actions Selected for EU Co-Funding Call for Proposals* (2014). Available at: https://op.europa.eu/en/publication-detail/-/publication/941eb798-ceb7-4269-bea7-c39405677ad1/language-en/format-PDF/source-240262429https://op.europa.eu/en/publication-detail/-/publication/941eb798-ceb7-4269-bea7-c39405677ad1/language-en/format-PDF/source-240262429 (Accessed November 05, 2021).
27. Aymé S, Rodwell C. The European Union Committee of Experts on Rare Diseases: Three Productive Years at the Service of the Rare Disease Community. *Orphanet J Rare Dis* (2014) 9:30. doi: 10.1186/1750-1172-9-30
28. European Union Committee of Experts on Rare Diseases. *Eucerd Core Recommendations on Rare Disease Patient Registration and Data Collection* (2013). Available at: https://rarediseases.org/wp-content/uploads/2015/12/EUCERD_Recommendations_RDRegistryDataCollection_adopted.pdfhttps://rarediseases.org/wp-content/uploads/2015/12/EUCERD_Recommendations_RDRegistryDataCollection_adopted.pdf (Accessed November 05, 2021).
29. European Commission Joint Research Centre. *Set of Common Data Elements for Rare Diseases Registration* (2017). Available at: https://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/CDS/EU_RD_Platform_CDS_Final.pdfhttps://eu-rd-platform.jrc.ec.europa.eu/sites/default/files/CDS/EU_RD_Platform_CDS_Final.pdf (Accessed November 05, 2021).
30. Opladen T, Gleich F, Kozich V, Scarpa M, Martinelli D, Schaefer F, et al. U-IMD: The First Unified European Registry for Inherited Metabolic Diseases. *Orphanet J Rare Dis* (2021) 16(1):95. doi: 10.1186/s13023-021-01726-3
31. Posset R, Garbade SF, Boy N, Burlina AB, Dionisi-Vici C, Dobbelaere D, et al. Transatlantic Combined and Comparative Data Analysis of 1095 Patients With Urea Cycle Disorders-A Successful Strategy for Clinical Research of Rare Diseases. *J Inherit Metab Dis* (2019) 42(1):93–106. doi: 10.1002/jimd.12031
32. Dhombres F, Bodenreider O. Interoperability Between Phenotypes in Research and Healthcare Terminologies–Investigating Partial Mappings Between HPO and SNOMED Ct. *J BioMed Semantics* (2016) 7:3. doi: 10.1186/s13326-016-0047-3
33. Brennenstuhl H, Kohlmüller D, Gramer G, Garbade SF, Syrbe S, Feyh P, et al. High Throughput Newborn Screening for Aromatic L-Amino-Acid Decarboxylase Deficiency by Analysis of Concentrations of 3-O-Methyldopa From Dried Blood Spots. *J Inherit Metab Dis* (2020) 43(3):602–10. doi: 10.1002/jimd.12208
34. Kölker S, Garcia-Cazorla A, Valayannopoulos V, Lund AM, Burlina AB, Sykut-Cegielska J, et al. The Phenotypic Spectrum of Organic Acidurias and Urea Cycle Disorders. Part 1: The Initial Presentation. *J Inherit Metab Dis* (2015) 38(6):1041–57. doi: 10.1007/s10545-015-9839-3
35. Rüegger CM, Lindner M, Ballhausen D, Baumgartner MR, Beblo S, Das A, et al. Cross-Sectional Observational Study of 208 Patients With Non-Classical Urea Cycle Disorders. *J Inherit Metab Dis* (2014) 37(1):21–30. doi: 10.1007/s10545-013-9624-0
36. Staufner C, Peters B, Wagner M, Alameer S, Barić I, Broué P, et al. Defining Clinical Subgroups and Genotype-Phenotype Correlations in NBAS-Associated Disease Across 110 Patients. *Genet Med* (2020) 22(3):610–21. doi: 10.1038/s41436-019-0698-4
37. Kuseyri Hübschmann O, Horvath G, Cortès-Saladelafont E, Yıldız Y, Mastrangelo M, Pons R, et al. Insights Into the Expanding Phenotypic Spectrum of Inherited Disorders of Biogenic Amines. *Nat Commun* (2021) 12(1):5529. doi: 10.1038/s41467-021-25515-5
38. Boy N, Mengler K, Thimm E, Schiergens KA, Marquardt T, Weinhold N, et al. Newborn Screening: A Disease-Changing Intervention for Glutaric Aciduria Type 1. *Ann Neurol* (2018) 83(5):970–9. doi: 10.1002/ana.25233
39. Hörster F, Baumgartner MR, Viardot C, Suormala T, Burgard P, Fowler B, et al. Long-Term Outcome in Methylmalonic Acidurias Is Influenced by the Underlying Defect (Mut0, Mut-, Cbla, Cblb). *Pediatr Res* (2007) 62(2):225–30. doi: 10.1203/PDR.0b013e3180a0325f
40. Hörster F, Tuncel AT, Gleich F, Plessl T, Froese SD, Garbade SF, et al. Delineating the Clinical Spectrum of Isolated Methylmalonic Acidurias: cblA and Mut. *J Inherit Metab Dis* (2021) 44(1):193–214. doi: 10.1002/jimd.12297
41. Kožich V, Sokolová J, Morris AAM, Pavlíková M, Gleich F, Kölker S, et al. Cystathionine β-Synthase Deficiency in the E-HOD Registry-Part I: Pyridoxine Responsiveness as a Determinant of Biochemical and Clinical Phenotype at Diagnosis. *J Inherit Metab Dis* (2021) 44(3):677–92. doi: 10.1002/jimd.12338
42. Mütze U, Henze L, Gleich F, Lindner M, Grünert SC, Spiekerkoetter U, et al. Newborn Screening and Disease Variants Predict Neurological Outcome in Isovaleric Aciduria. *J Inherit Metab Dis* (2021) 44(4):857–70. doi: 10.1002/jimd.12364
43. Enns GM, Berry SA, Berry GT, Rhead WJ, Brusilow SW, Hamosh A. Survival After Treatment With Phenylacetate and Benzoate for Urea-Cycle Disorders. *N Engl J Med* (2007) 356(22):2282–92. doi: 10.1056/NEJMoa066596
44. Posset R, Garcia-Cazorla A, Valayannopoulos V, Teles EL, Dionisi-Vici C, Brassier A, et al. Age at Disease Onset and Peak Ammonium Level Rather

Than Interventional Variables Predict the Neurological Outcome in Urea Cycle Disorders. *J Inherit Metab Dis* (2016) 39(5):661–72. doi: 10.1007/s10545-016-9938-9
45. Buerger C, Garbade SF, Dietrich Alber F, Waisbren SE, McCarter R, Kölker S, et al. Impairment of Cognitive Function in Ornithine Transcarbamylase Deficiency Is Global Rather Than Domain-Specific and Is Associated With Disease Onset, Sex, Maximum Ammonium, and Number of Hyperammonemic Events. *J Inherit Metab Dis* (2019) 42(2):243–53. doi: 10.1002/jimd.12013
46. Posset R, Gropman AL, Nagamani SCS, Burrage LC, Bedoyan JK, Wong D, et al. Impact of Diagnosis and Therapy on Cognitive Function in Urea Cycle Disorders. *Ann Neurol* (2019) 86(1):116–28. doi: 10.1002/ana.25492
47. Guthrie R, Susi A. A Simple Phenylalanine Method for Detecting Phenylketonuria in Large Populations of Newborn Infants. *Pediatrics* (1963) 32:338–43. doi: 10.1542/peds.32.3.338
48. Therrell BL, Padilla CD, Loeber JG, Kneisser I, Saadallah A, Borrajo GJ, et al. Current Status of Newborn Screening Worldwide: 2015. *Semin Perinatol* (2015) 39(3):171–87. doi: 10.1053/j.semperi.2015.03.002
49. Loeber JG, Platis D, Zetterström RH, Almashanu S, Boemer F, Bonham JR, et al. Neonatal Screening in Europe Revisited: An ISNS Perspective on the Current State and Developments Since 2010. *Int J Neonatal Screen* (2021) 7 (1):1–21. doi: 10.3390/ijns7010015
50. Heringer J, Valayannopoulos V, Lund AM, Wijburg FA, Freisinger P, Barić I, et al. Impact of Age at Onset and Newborn Screening on Outcome in Organic Acidurias. *J Inherit Metab Dis* (2016) 39(3):341–53. doi: 10.1007/s10545-015-9907-8
51. Mütze U, Garbade SF, Gramer G, Lindner M, Freisinger P, Grunert SC, et al. Long-Term Outcomes of Individuals With Metabolic Diseases Identified Through Newborn Screening. *Pediatrics* (2020) 146(5):1–11. doi: 10.1542/peds.2020-0444
52. Boy N, Mengler K, Heringer-Seifert J, Hoffmann GF, Garbade SF, Kölker S. Impact of Newborn Screening and Quality of Therapy on the Neurological Outcome in Glutaric Aciduria Type 1: A Meta-Analysis. *Genet Med* (2021) 23 (1):13–21. doi: 10.1038/s41436-020-00971-4
53. Mütze U, Walter M, Keller M, Gramer G, Garbade SF, Gleich F, et al. Health Outcomes of Infants With Vitamin B(12) Deficiency Identified by Newborn Screening and Early Treated. *J Pediatr* (2021) 235:42–8. doi: 10.1016/j.jpeds.2021.02.009
54. Bjugstad KB, Goodman SI, Freed CR. Age at Symptom Onset Predicts Severity of Motor Impairment and Clinical Outcome of Glutaric Acidemia Type 1. *J Pediatr* (2000) 137(5):681–6. doi: 10.1067/mpd.2000.108954
55. Boy N, Mühlhausen C, Maier EM, Heringer J, Assmann B, Burgard P, et al. Proposed Recommendations for Diagnosing and Managing Individuals With Glutaric Aciduria Type I: Second Revision. *J Inherit Metab Dis* (2017) 40 (1):75–101. doi: 10.1007/s10545-016-9999-9
56. Märtner EMC, Maier EM, Mengler K, Thimm E, Schiergens KA, Marquardt T, et al. Impact of Interventional and Non-Interventional Variables on Anthropometric Long-Term Development in Glutaric Aciduria Type 1: A National Prospective Multi-Centre Study. *J Inherit Metab Dis* (2021) 44 (3):629–38. doi: 10.1002/jimd.12335
57. Strauss KA, Williams KB, Carson VJ, Poskitt L, Bowser LE, Young M, et al. Glutaric Acidemia Type 1: Treatment and Outcome of 168 Patients Over Three Decades. *Mol Genet Metab* (2020) 131(3):325–40. doi: 10.1016/j.ymgme.2020.09.007
58. Molema F, Gleich F, Burgard P, van der Ploeg AT, Summar ML, Chapman KA, et al. Evaluation of Dietary Treatment and Amino Acid Supplementation in Organic Acidurias and Urea-Cycle Disorders: On the Basis of Information From a European Multicenter Registry. *J Inherit Metab Dis* (2019) 42 (6):1162–75. doi: 10.1002/jimd.12066
59. Kölker S, Christensen E, Leonard JV, Greenberg CR, Burlina AB, Burlina AP, et al. Guideline for the Diagnosis and Management of Glutaryl-CoA Dehydrogenase Deficiency (Glutaric Aciduria Type I). *J Inherit Metab Dis* (2007) 30(1):5–22. doi: 10.1007/s10545-006-0451-4
60. Kölker S, Christensen E, Leonard JV, Greenberg CR, Boneh A, Burlina AB, et al. Diagnosis and Management of Glutaric Aciduria Type I–revised Recommendations. *J Inherit Metab Dis* (2011) 34(3):677–94. doi: 10.1007/s10545-011-9289-5
61. Boy N, Heringer J, Brackmann R, Bodamer O, Seitz A, Kölker S, et al. Extrastriatal Changes in Patients With Late-Onset Glutaric Aciduria Type I Highlight the Risk of Long-Term Neurotoxicity. *Orphanet J Rare Dis* (2017) 12(1):77. doi: 10.1186/s13023-017-0612-6
62. Kölker S, Garbade SF, Greenberg CR, Leonard JV, Saudubray JM, Ribes A, et al. Natural History, Outcome, and Treatment Efficacy in Children and Adults With Glutaryl-CoA Dehydrogenase Deficiency. *Pediatr Res* (2006) 59 (6):840–7. doi: 10.1203/01.pdr.0000219387.79887.86
63. Heringer J, Boy SP, Ensenauer R, Assmann B, Zschocke J, Harting I, et al. Use of Guidelines Improves the Neurological Outcome in Glutaric Aciduria Type I. *Ann Neurol* (2010) 68(5):743–52. doi: 10.1002/ana.22095
64. Boy N, Mohr A, Garbade SF, Freisinger P, Heringer-Seifert J, Seitz A, et al. Subdural Hematoma in Glutaric Aciduria Type 1: High Excreters Are Prone to Incidental SDH Despite Newborn Screening. *J Inherit Metab Dis* (2021) 44 (6):1343–52. doi: 10.1002/jimd.12436
65. Pfeil J, Listl S, Hoffmann GF, Kölker S, Lindner M, Burgard P. Newborn Screening by Tandem Mass Spectrometry for Glutaric Aciduria Type 1: A Cost-Effectiveness Analysis. *Orphanet J Rare Dis* (2013) 8:167. doi: 10.1186/1750-1172-8-167
66. Märtner EMC, Thimm E, Guder P, Schiergens KA, Rutsch F, Roloff S, et al. The Biochemical Subtype Is a Predictor for Cognitive Function in Glutaric Aciduria Type 1: A National Prospective Follow-Up Study. *Sci Rep* (2021) 11 (1):19300. doi: 10.1038/s41598-021-98809-9
67. Strauss KA, Puffenberger EG, Robinson DL, Morton DH. Type I Glutaric Aciduria, Part 1: Natural History of 77 Patients. *Am J Med Genet C Semin Med Genet* (2003) 121c(1):38–52. doi: 10.1002/ajmg.c.20007
68. Strauss KA, Brumbaugh J, Duffy A, Wardley B, Robinson D, Hendrickson C, et al. Safety, Efficacy and Physiological Actions of a Lysine-Free, Arginine-Rich Formula to Treat Glutaryl-CoA Dehydrogenase Deficiency: Focus on Cerebral Amino Acid Influx. *Mol Genet Metab* (2011) 104(1-2):93–106. doi: 10.1016/j.ymgme.2011.07.003
69. Höliner I, Simma B, Reiter A, Sass JO, Zschocke J, Huemer M. Compliance to Clinical Guidelines Determines Outcome in Glutaric Aciduria Type I in the Era of Newborn Screening. *Klin Padiatr* (2010) 222(1):35–7. doi: 10.1055/s-0029-1239525
70. Häberle J, Burlina A, Chakrapani A, Dixon M, Karall D, Lindner M, et al. Suggested Guidelines for the Diagnosis and Management of Urea Cycle Disorders: First Revision. *J Inherit Metab Dis* (2019) 42(6):1192–230. doi: 10.1002/jimd.12100
71. Häberle J, Boddaert N, Burlina A, Chakrapani A, Dixon M, Huemer M, et al. Suggested Guidelines for the Diagnosis and Management of Urea Cycle Disorders. *Orphanet J Rare Dis* (2012) 7:32. doi: 10.1186/1750-1172-7-32
72. Baumgartner MR, Hörster F, Dionisi-Vici C, Haliloglu G, Karall D, Chapman KA, et al. Proposed Guidelines for the Diagnosis and Management of Methylmalonic and Propionic Acidemia. *Orphanet J Rare Dis* (2014) 9:130. doi: 10.1186/s13023-014-0130-8
73. Forny P, Hörster F, Ballhausen D, Chakrapani A, Chapman KA, Dionisi-Vici C, et al. Guidelines for the Diagnosis and Management of Methylmalonic Acidaemia and Propionic Acidaemia: First Revision. *J Inherit Metab Dis* (2021) 44(3):566–92. doi: 10.1002/jimd.12370
74. Huemer M, Diodato D, Schwahn B, Schiff M, Bandeira A, Benoist JF, et al. Guidelines for Diagnosis and Management of the Cobalamin-Related Remethylation Disorders Cblc, Cbld, Cble, Cblf, Cblg, cblJ and MTHFR Deficiency. *J Inherit Metab Dis* (2017) 40(1):21–48. doi: 10.1007/s10545-016-9991-4
75. Huemer M, Diodato D, Martinelli D, Olivieri G, Blom H, Gleich F, et al. Phenotype, Treatment Practice and Outcome in the Cobalamin-Dependent Remethylation Disorders and MTHFR Deficiency: Data From the E-HOD Registry. *J Inherit Metab Dis* (2019) 42(2):333–52. doi: 10.1002/jimd.12041
76. Keller R, Chrastina P, Pavlíková M, Gouveia S, Ribes A, Kölker S, et al. Newborn Screening for Homocystinurias: Recent Recommendations Versus Current Practice. *J Inherit Metab Dis* (2019) 42(1):128–39. doi: 10.1002/jimd.12034
77. Morris AA, Kožich V, Santra S, Andria G, Ben-Omran TI, Chakrapani AB, et al. Guidelines for the Diagnosis and Management of Cystathionine Beta-Synthase Deficiency. *J Inherit Metab Dis* (2017) 40(1):49–74. doi: 10.1007/s10545-016-9979-0
78. Opladen T, López-Laso E, Cortès-Saladelafont E, Pearson TS, Sivri HS, Yildiz Y, et al. Consensus Guideline for the Diagnosis and Treatment of Tetrahydrobiopterin (BH(4)) Deficiencies. *Orphanet J Rare Dis* (2020) 15 (1):126. doi: 10.1186/s13023-020-01379-8

79. Wassenberg T, Molero-Luis M, Jeltsch K, Hoffmann GF, Assmann B, Blau N, et al. Consensus Guideline for the Diagnosis and Treatment of Aromatic L-Amino Acid Decarboxylase (AADC) Deficiency. *Orphanet J Rare Dis* (2017) 12(1):12. doi: 10.1186/s13023-016-0522-z
80. Wilkinson MD, Dumontier M, Aalbersberg IJ, Appleton G, Axton M, Baak A, et al. The FAIR Guiding Principles for Scientific Data Management and Stewardship. *Sci Data* (2016) 3:160018. doi: 10.1038/sdata.2016.18
81. European Medicines Agency. *Patient Registry Initiative- Strategy and Mandate of the Cross-Committee Task Force* (2017). Available at: https://www.ema.europa.eu/en/documents/other/patient-registry-initiative-strategy-mandate-cross-committee-task-force_en.pdfhttps://www.ema.europa.eu/en/documents/other/patient-registry-initiative-strategy-mandate-cross-committee-task-force_en.pdf (Accessed November 05, 2021).
82. European Medicines Agency. *Initiative for Patient Registries Strategy and Pilot Phase* (2015). Available at: https://www.ema.europa.eu/en/documents/other/initiative-patient-registries-strategy-pilot-phase_en.pdfhttps://www.ema.europa.eu/en/documents/other/initiative-patient-registries-strategy-pilot-phase_en.pdf (Accessed November 05, 2021).
83. Derks TGJ, Rodriguez-Buritica DF, Ahmad A, de Boer F, Couce ML, Grünert SC, et al. Glycogen Storage Disease Type Ia: Current Management Options, Burden and Unmet Needs. *Nutrients* (2021) 13(11):3828. doi: 10.3390/nu13113828
84. Ali, Salma R, Bryce J, Kodra Y, Taruscio D, Persani L, et al. The Quality Evaluation of Rare Disease Registries—An Assessment of the Essential Features of a Disease Registry. Int. J. Environ. Res. *Public Health* (2021) 18:11968. doi: 10.3390/ijerph182211968

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The Role of GLP-1 Signaling in Hypoglycemia due to Hyperinsulinism

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Incretin hormones play an important role in the regulation of glucose homeostasis through their actions on the beta cells and other tissues. Glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) are the two main incretins and are secreted by enteroendocrine L- and K-cells, respectively. New evidence suggests that incretin hormones, particularly GLP-1, play a role in the pathophysiology of hyperinsulinemic hypoglycemia. In individuals with acquired hyperinsulinemic hypoglycemia after gastrointestinal surgery, including Nissen fundoplication and gastric bypass surgery, the incretin response to a meal is markedly increased and antagonism of the GLP-1 receptor prevents the hyperinsulinemic response. In individuals with congenital hyperinsulinism due to inactivating mutations in the genes encoding the beta cell K_{ATP} channels, the GLP-1 receptor antagonist, exendin-(9-39), increases fasting plasma glucose and prevents protein-induced hypoglycemia. Studies in human and mouse islets lacking functional K_{ATP} channels have demonstrated that the effect on plasma glucose is at least in part mediated by inhibition of insulin secretion resulting from lower cytoplasmic cAMP levels. The understanding of the role of incretin hormones in the pathophysiology of hyperinsulinemic hypoglycemia is important for the exploration of the GLP-1 receptor as a therapeutic target for these conditions. In this article, we will review incretin physiology and evidence supporting a role of the incretin hormones in the pathophysiology of hyperinsulinemic hypoglycemia, as well as results from proof-of concept studies exploring a therapeutic approach targeting the GLP-1 receptor to treat hyperinsulinemic hypoglycemia.

Keywords: incretin, GLP-1, GIP, hyperinsulinism, dumping syndrome, bariatric surgery, fundoplication

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INTRODUCTION

Incretins are hormones secreted by intestinal cells in response to ingested nutrients that play a role in the regulation of glucose homeostasis. Glucagon-like-peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) are the two main incretins. Incretins effects on glucose homeostasis are mediated by several mechanisms, the most prominent being potentiation of insulin secretion. Effects of endogenous GLP-1 have been better characterized using the receptor antagonist exendin-(9-39). In this article, we will review incretin physiology and evidence supporting a role of GLP-1 in

the pathophysiology of hyperinsulinemic hypoglycemia, as well as results from proof-of concept studies exploring a therapeutic approach targeting the GLP-1 receptor to treat hyperinsulinemic hypoglycemia.

INCRETIN PHYSIOLOGY

GLP-1 is made and released by the enteroendocrine L-cells, which are found throughout the small and large intestines, with higher concentration in the ileum. GIP is secreted by the enteroendocrine K-cells concentrated in the proximal intestines, including the duodenum and jejunum (1, 2). Incretin secretion is stimulated by nutrient ingestion, the most potent activators being carbohydrate and lipid ingestion; protein is a weaker stimulus for incretin secretion (3). Levels of incretins increase rapidly after food administration *via* both neural and endocrine mediated factors (4). The secretion of GIP from K-cells is dependent on nutrient absorption, whereas L-cells can secrete GLP-1 triggered by glucose in the intestinal lumen. This observation is supported by studies in patients with disorders affecting gut absorption demonstrating a significant decrease in plasma GIP levels following a meal and normal to elevated GLP-1 levels (5). This phenomenon, however, can also be explained by increased glucose distribution to distal intestinal cells in patients with malabsorption, leading to higher luminal glucose concentrations in the distal intestines where GLP-1 secreting L-cells are highly concentrated (6). GLP-1 plasma levels rise within minutes of oral nutrient ingestion, suggesting that neural factors and taste receptors play a role in stimulating its secretion, in addition to direct contact with the enteroendocrine cells of the intestines (7). GIP and GLP-1 plasma levels are low in the fasting state, and increase post-prandially; GIP concentrations are higher than GLP-1 concentrations in both fasting and fed state (2). GIP plasma concentrations peak 30 minutes after a meal, and plasma GLP-1 rises within a few minutes and levels remain high for several hours after a meal. Both incretins are hydrolyzed by the enzyme dipeptidyl-peptidase 4 (DPP-IV).

Incretins act on G-protein coupled receptors on the beta cells of the pancreas, leading to a cAMP mediated pathway that ultimately results in insulin secretion (3). New research has also shown a separate pathway where low concentrations of GLP-1 lead to increased intracellular calcium and insulin secretion independent of the cAMP pathway (8) (**Figure 1**). In addition to its direct effects on beta cells, GIP also leads to increased glucagon secretion, and indirectly results in increased insulin secretion *via* communication between the alpha and beta cells of the pancreas (9). GLP-1 suppresses glucagon secretion *via* complex mechanisms, including stimulation of somatostatin secretion, which inhibits glucagon secretion, and indirectly *via* stimulation of insulin production, which then inhibits glucagon release (10).

Incretin action plays a large role on post-prandial secretion of insulin, and may account for up to 70% of hormone release (6). Thus, gut absorption of glucose leads to significantly greater rise in plasma insulin when compared to intravenous administration of glucose, due to incretin mediated insulin secretion (4), a phenomenon known as the "incretin effect". Incretins also promote pancreatic beta cell proliferation and cell survival by decreasing apoptosis (6, 11). GLP-1 increases insulin secretion and synthesis as well as increases insulin sensitivity, induces beta cell growth, and inhibits glucagon secretion (1). In addition to post-prandial effects, GLP-1 suppresses glucagon in the fasting state, and can inhibit duodenal motility, leading to lower fasting plasma glucose (12). This suggests incretin effects that are independent of insulin action, as insulin levels remain low to undetectable in this state.

Both incretins have effects outside the pancreas. GLP-1 effects include slowing gastric emptying, increasing satiety, as well as cardioprotective effects (3, 13). In addition to secretion by intestinal cells, GLP-1 is produced in the central nervous system and can cross the blood brain barrier. Evidence suggests that GLP-1 has neuroprotective effects in addition to having effects on appetite regulation by decreasing appetite and increasing satiety (14, 15). GIP also has extra-pancreatic effects, including, stimulation of lipogenesis and stimulation of bone formation *via* inducing osteoblast activity and reduction of bone resorption *via* inhibition of osteoclasts (3) (**Figure 1**). The GLP-1 receptor antagonist, exendin-(9-39), has been used to demonstrate physiologic effects of endogenous GLP-1 in human studies. Exendin-(9-39) is a truncated form of exendin-4, a GLP-1 receptor agonist, isolated from the saliva of the gila monster. Exendin-(9-39) has been found to competitively bind to the human GLP-1 receptor (16). Some studies have shown that exendin-(9-39) also binds the GIP receptor (17, 18), however, Schirra and colleagues have demonstrated that doses of exendin-(9-39) that completely antagonize the insulinotropic effects of GLP-1 do not alter the insulinotropic activity of GIP *in vivo* in humans (16). Administration of exendin-(9-39) in healthy human subjects has been shown to decrease insulin secretion and increase glucose levels following meals, increase glucagon and glucose levels in the fasting state, and decrease insulin action with increased glucose following intravenous glucose administration (12, 19, 20), demonstrating *in vivo* that endogenous GLP-1 plays an important role on maintaining glucose homeostasis both in the fasting and in the postprandial state through its effects on pancreatic islets.

Because of their glucose lowering effects, therapies that enhance incretin actions through inhibiting degradation of endogenous incretins or through activation of the GLP-1 receptor are now in use for the treatment of type 2 diabetes (21, 22). In addition, pharmacologic doses of GLP-1 analogues have potent weight loss effects and are now in the market (23, 24). These pharmacologic studies have shown that these therapies are safe and well-tolerated and that they are not associated with overt hypoglycemia. However, GLP-1 administration has been shown to result in hypoglycemia when concomitantly administered with intravenous dextrose (25) or with sulfonylureas (26). What these two conditions may have in common is that the beta cells are in a depolarized state, thus, the glucose-dependency of GLP-1 insulinotropic effects is lost. Here we will discuss two pathologic conditions were GLP-1 has been associated with hypoglycemia, in congenital hyperinsulinism

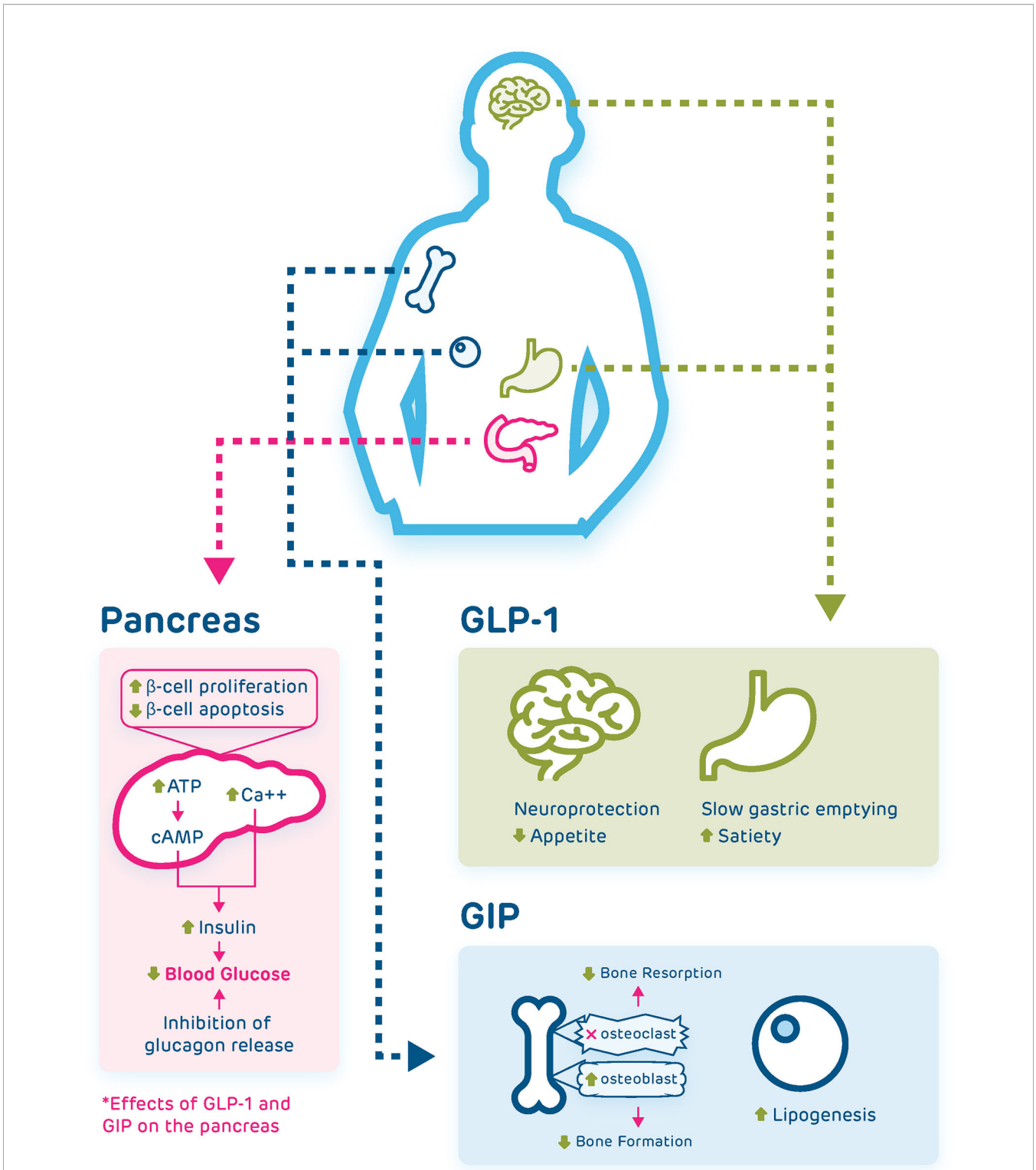

FIGURE 1 | Pancreatic and extrapancreatic effects of GLP-1 and GIP. Both incretins act on the beta cells of the pancreas and lead to reduction of blood glucose *via* several mechanisms, including increasing intracellular calcium, induction of a cAMP mediated pathway, and inhibition of glucagon release. GLP-1 also acts on the brain where it has neuroprotective effects and suppresses appetite, and on the stomach where it leads to slowed gastric emptying and increased satiety. GIP acts on the bone where it inhibits osteoclast action and promotes osteoblast action, leading to increased bone formation, and acts on fat cells to induce lipogenesis.

due to inactivating mutations in the K_{ATP} channels and in postprandial hypoglycemia after gastrointestinal surgery, where the delivery of nutrients to the small intestine is altered resulting in both hyperglycemia and an exaggerated secretion of GLP-1. In both conditions, one would hypothesized that the glucose-dependency of GLP-1 is also lost given the lack of functional K_{ATP} channels in the first, and the concomitant hyperglycemia in the second.

ROLE OF GLP-1 IN CONGENITAL HYPERINSULINISM

Congenital hyperinsulinism is the most common cause of persistent hypoglycemia in neonates, infants, and children. Congenital hyperinsulinism is a heterogeneous condition in regards to genotype and phenotype. Over 12 different genetic loci have been associated with congenital hyperinsulinism but the most common genetic cause, which accounts for up to 60% of cases (27), are inactivating mutations in *ABCC8* and *KCNJ11*, which encode the two subunits of the beta cell K_{ATP} channel. Histologically, congenital hyperinsulinism can be diffuse, where all beta cells are affected, or focal, where there is a focal area of adenomatosis (28). Phenotypically, there are differences on triggers of hypoglycemia (fasting, protein consumption, exercise) and responsiveness to treatment, among the different types of hyperinsulinism (28).

Plasma incretin concentrations in both the fasting and fed state have been measured in patients with different forms of congenital hyperinsulinism. A study reported that there was no difference in post-prandial plasma incretin concentrations between diffuse and focal cases, and no difference in baseline plasma incretin concentration between focal, diffuse, and transient hyperinsulinism (29). Patients with atypical hyperinsulinism (defined as having no identified genetic mutation and with mosaic histomorphology) had significantly higher post-prandial plasma GLP-1 concentration when compared to other forms of hyperinsulinism (29). The study authors proposed that increased insulin levels in atypical hyperinsulinism may be driven by increased incretin action, specifically GLP-1, and propose that GLP-1 can be utilized as a biomarker to help identify and diagnose atypical hyperinsulinism (29), however, these findings need to be replicated in a larger population.

Studies in a mouse model of K_{ATP} hyperinsulinism, the most common and severe genetic subtype of hyperinsulinism, have shown that the GLP-1 receptor antagonist exendin-(9-39) significantly increases fasting plasma glucose and decreases the insulin to glucose ratio on the fasting state, effectively reversing the hyperinsulinemic hypoglycemia phenotype. These effects were mediated by reduction in islet cAMP concentration, as demonstrated by experiments *in vitro* in isolated *Sur1*$^{-/-}$ mouse islets (30). In addition to the effects of exendin-(9-39) on baseline islet cAMP and insulin secretion, exendin-(9-39) inhibited amino acid-stimulated insulin secretion, a key phenotypic feature of hyperinsulinism due to inactivating mutations in the K_{ATP} channels where consumption of protein leads to insulin secretion and hypoglycemia (30, 31).

The findings from mouse islets, have been replicated in human islets isolated from the pancreas of infants with diffuse K_{ATP} hyperinsulinism. Exendin-(9-39) effectively inhibited amino acid-stimulated insulin secretion from these islets. Furthermore, in adolescents and adults with K_{ATP} hyperinsulinism, a continuous intravenous infusion of exendin-(9-39) resulted in higher fasting plasma glucose concentrations and lower insulin to glucose ratio; plasma glucagon concentration was unaffected by exendin-(9-39) in this study. These studies suggest that GLP-1 and its receptor play a role in the pathophysiology of congenital hyperinsulinism and that targeting the GLP-1 receptor may be an effective treatment approach for this condition (32). In more recent studies, we have shown that exendin-(9-39) prevents both fasting and protein-induced hypoglycemia in children with K_{ATP} hyperinsulinism and decreases glucose infusion rate requirements in infants with K_{ATP} hyperinsulinism (33–35). Because of these promising results, exendin-(9-39) was granted breakthrough therapy designation for the treatment of congenital hyperinsulinism by the Food and Drug Administration, and studies to evaluate efficacy and safety of multiple dose regimens are under development (36).

ROLE OF GLP-1 IN HYPERINSULINEMIC HYPOGLYCEMIA SECONDARY TO GASTRIC SURGERY

Plasma glucose homeostasis, particularly in the post-prandial state is altered after gastric surgery, including gastrectomy, fundoplication, and bariatric procedures (37). Severe postprandial hypoglycemia following an exaggerated insulin response to meals has been recognized as a consequence of these procedures. Incretins contribute to the post-prandial hypoglycemia *via* increased GLP-1 release potentiating insulin secretion.

GLP-1 in Post-Prandial Hypoglycemia Following Fundoplication

Post-prandial hypoglycemia, also known as late dumping syndrome, is a relatively common phenomenon seen in children who undergo surgical procedures altering the anatomy or function of the gastrointestinal tract, particularly after Nissen Fundoplication or variants thereof. Approximately 25% of children who undergo Nissen Fundoplication experience post-prandial hypoglycemia (38). Nutrient ingestion, particularly if given through a gastrostomy, in children who underwent Nissen Fundoplication leads to rapid increase in plasma glucose as well as plasma insulin followed by hypoglycemia; this may be, in part, due to exaggerated GLP-1 secretion (39, 40). One study analyzed risk factors for developing dumping syndrome following fundoplication in children. Risk factors for dumping syndrome that were identified included fundoplication surgery within 1 year of age, presence of severe scoliosis, microgastria, and major cardiac abnormality (41).

Children with post-prandial hypoglycemia after Nissen fundoplication exhibit higher GLP-1 and insulin plasma concentrations, and lower plasma glucose nadir in response to an oral glucose tolerance test than controls. The authors proposed that the increased GLP-1 levels potentiated the insulin surge and contributed to the subsequent hypoglycemia. The children that participated in the study had appropriate suppression of insulin secretion in response to hypoglycemia and had a normal fasting tolerance, arguing against an underlying disorder of insulin secretion (39). Alterations on the delivery of nutrients to the small intestine resulting from the fundoplication, which decreases the accommodating capacity of the stomach, may explain the rapid rise in glucose and enhanced GLP-1 release.

In order to better characterize the role of endogenous GLP-1 in post-prandial hypoglycemia secondary to fundoplication, the insulin response to a standardized mixed meal in affected children was measured with concomitant administration of exendin-(9-39) or vehicle through a continuous intravenous infusion. This study found that the insulin surge following the meal was blunted with the administration of the GLP-1 antagonist, suggesting that GLP-1 plays an important role in the exaggerated insulin response seen in post-prandial hypoglycemia in children who had undergone a fundoplication (42). Glucagon concentration was higher during administration of exendin-(9-39) versus the vehicle condition in this study (42).

GLP-1 in Post-Prandial Hypoglycemia Following Bariatric Surgery

A similar phenomenon of post-prandial hyperinsulinemic hypoglycemia is seen in adult patients after weight loss procedures, gastrectomy and Roux-en-Y gastric bypass, in particular; this is commonly referred to as dumping syndrome. Approximately 25% of patients who undergo total gastrectomy surgery experience subsequent dumping syndrome (43). Post-prandial hyperinsulinemic hypoglycemia in gastric bypass patients ameliorates with nutrient administration *via* the stomach (using a gastrostomy tube into the remnant stomach), rather than *via* the bypassed gastrointestinal tract. Post-prandial levels of insulin and GLP-1 normalized with nutrient administration into the stomach rather than the gastric bypass route, suggesting that altered nutrient delivery to the intestines contributes to dumping syndrome (44).

To better understand the timing of hormone secretion in relation to hypoglycemia in dumping syndrome, glucose, insulin, GLP-1, and norepinephrine levels were measured following a meal in patients with dumping syndrome, and compared to levels and timing of peak in control patients. Plasma insulin concentration peaked at 60 minutes in patients with dumping syndrome, and at 90 minutes in control patients. Plasma GLP-1 concentration peaked earlier and were higher in dumping syndrome patients; levels peaked at 20 minutes in patients with dumping syndrome and 30 minutes in control patients. There was a significant inverse correlation between plasma GLP-1 concentration shortly after a meal and plasma glucose concentration 2 hours post-prandially, coinciding with the plasma glucose nadir. Plasma glucagon was also measured in this study; baseline glucagon concentrations were similar between controls and subjects with dumping syndrome, and the glucagon levels peaked and remained elevated after 20 minutes in subjects with dumping syndrome. The study authors concluded that reactive hypoglycemia in dumping syndrome following bariatric surgery is caused by increased GLP-1 concentration potentiating insulin release (45).

Another similar study measured plasma incretin, insulin, and glucose concentration in gastrectomy patients compared to controls. In response to an oral glucose load, plasma GLP-1 concentration was increased in post-gastrectomy patients, when compared to controls with unaltered intestinal anatomy. There was a statistically significant correlation between GLP-1 increase and the presence of dumping syndrome. Plasma GLP-1 concentration peaked at 15 minutes post glucose load, and insulin peaked at 30 minutes, suggesting GLP-1 potentiated or triggered insulin secretion. The study found an inverse relation between the emptying time from the gastric pouch, and plasma GLP-1 and insulin concentration with the meal, indicating that quicker transit time led to increases in insulin and GLP-1 plasma concentration and subsequent post-prandial hypoglycemia (43).

Exendin-(9-39) given as an intravenous infusion to adult individuals with post-prandial hypoglycemia after gastric bypass surgery led to a blunted insulin response, suggesting that GLP-1 is responsible for the exaggerated insulin secretion in dumping syndrome (46). Exendin-(9-39) has shown to be effective in preventing hyperinsulinemic hypoglycemia in bariatric surgery patients, and may have therapeutic implications in this patient population (47, 48). One study assessed the effect of exendin-(9-39) administered as a subcutaneous injection in treating hypoglycemia following bariatric surgery by measuring glucose nadir and insulin peak during a mixed meal tolerance test, and by assessing glycemic control using home continuous glucose monitoring. Incidence of hypoglycemia measured by continuous glucose monitor and number of hypoglycemic events were decreased in the subjects treated with exendin-(9-39). There was a statistically significant increase in glucose nadir and decrease in insulin peak during the mixed meal tolerance test (49). Another study found a reduction in symptomatic hypoglycemic events, as well as decreased insulin peak and increased glucose nadir during oral glucose tolerance tests in subjects receiving exendin-(9-39) (50).

CONCLUSIONS

The importance of incretin hormones on the regulation of glucose homeostasis through pancreatic and extra-pancreatic actions is well established. Because of their glucose lowering effects, therapies that enhance incretin actions through inhibiting degradation of endogenous incretins or through activation of the GLP-1 receptor are now in use for the treatment of type 2 diabetes. The recognition of a role for GLP-1 in the pathophysiology of congenital and acquired hyperinsulinemic hypoglycemia has brought forward the possibility of targeting

the GLP-1 receptor for the treatment of this condition and promising progress has been made to this end.

AUTHOR CONTRIBUTIONS

MD performed literature review and wrote the manuscript. DDDL reviewed literature and edited the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Campbell JE, Drucker DD. Pharmacology, Physiology, and Mechanisms of Incretin Hormone Action. *Cell Metab* (2013) 17(6):819–37. doi: 10.1016/j.cmet.2013.04.008
2. Nauck MA, Meier JJ. Incretin Hormones: Their Role in Health and Disease. *Diabetes Obes Metab* (2018) 20:5–21. doi: 10.1111/dom.13129
3. Phillips LK, Prins JB. Update on Incretin Hormones. *Ann N Y Acad Sci* (2012) 1243(1):1–20. doi: 10.1111/j.1749-6632.2012.06491.x
4. Drucker DD. The Biology of Incretin Hormones. *Cell Metab* (2006) 3(3):153–65. doi: 10.1016/j.cmet.2006.01.004
5. Besterman HS, Bloom SR, Sarson DL, Blackburn AM, Johnston DI, Patel HR, et al. Gut Hormone Profile in Coeliac Disease. *Lancet* (1978) 1:785–8. doi: 10.1016/S0140-6736(78)92994-X
6. Diakogiannaki E, Gribble FM, Reimann F. Nutrient Detection by Incretin Hormone Secreting Cells. *Physiol Behav* (2012) 106(3):387–93. doi: 10.1016/j.physbeh.2011.12.001
7. Gautier JF, Choukem SP, Girard J. Physiology of Incretins (GIP and GLP-1) and Abnormalities in Type 2 Diabetes. *Diabetes Metab* (2008) 34:65–72. doi: 10.1016/S1262-3636(08)73397-4
8. Kaku K. New Concept of the Glucagon-Like Peptide-1 Signaling Pathway on Pancreatic Insulin Secretion. *J Diabetes Investig* (2020) 11(2):265–7. doi: 10.1111/jdi.13136
9. El K, Gray SM, Capozzi ME, Knuth ER, Jin E, Svendsen B, et al. GIP Mediates the Incretin Effect and Glucose Tolerance by Dual Actions on Alpha and Beta Cells. *Sci Adv* (2021) 7:1–10. doi: 10.1126/sciadv.abf1948
10. Müller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, et al. Glucagon-Like-Peptide 1 (GLP-1). *Mol Metab* (2019) 30:72–130. doi: 10.1016/j.molmet.2019.09.010
11. Lee Y-S, Jun H-S. Anti-Diabetic Actions of Glucagon-Like Peptide-1 on Pancreatic Beta-Cells. *Metabolism* (2014) 63(1):9–19. doi: 10.1016/j.metabol.2013.09.010
12. Schirra J, Nicholaus M, Roggel R, Katschinski M, Storr M, Woerle HJ, et al. Endogenous Glucagon-Like Peptide 1 Controls Endocrine Pancreatic Secretion and Antro-Pyloro-Duodenal Motility in Humans. *Gut* (2006) 55:243–51. doi: 10.1136/gut.2004.059741
13. Ussher JR, Drucker DJ. Cardiovascular Biology of the Incretin System. *Endocr Rev* (2012) 33(2):187–215. doi: 10.1210/er.2011-1052
14. During MJ, Cao L, Zuzga DS, Francis SJ, Fitzsimons HL, Jiao X, et al. Glucagon-Like Peptide-1 Receptor Is Involved in Learning and Neuroprotection. *Nat Med* (2003) 9:1173–9. doi: 10.1038/nm919
15. Kreiger J-P. Intestinal Glucagon-Like Peptide-1 Effects on Food Intake: Physiological Relevance and Emerging Mechanisms. *Peptides* (2020) 131:170342. doi: 10.1016/j.peptides.2020.170342
16. Schirra J, Sturm K, Leicht P, Arnold R, Göke B, Katschinski M, et al. Exendin (9-39)Amide Is an Antagonist of Glucagon-Like Peptide-1(7-36)Amide in Humans. *J Clin Invest* (1998) 101(7):1421–30. doi: 10.1172/JCI1349
17. Gremlich S, Porret A, Hani EH, Cherif D, Vionnet N, Froguel P, et al. Cloning, Functional Expression, and Chromosomal Localization of the Human Pancreatic Islet Glucose-Dependent Insulinotropic Polypeptide Receptor. *Diabetes* (1995) 44:1202–8. doi: 10.2337/diabetes.44.10.1202
18. Gault VA, O'Harte FPM, Harriot P, Mooney MH, Green BD, Flatt PR. Effects of the Novel (Pro^3)GIP Antagonist and Exendin(9-39)Amide on GIP- and GLP-1 Induced Cyclic AMP Generation, Insulin Secretion and Postprandial Insulin Release in Obese Diabetic (*Ob/Ob*) Mice: Evidence that GIP Is the Major Physiological Incretin. *Diabetologia* (2003) 46:222–30. doi: 10.1007/s00125-002-1028-x
19. Schirra J, Nicolaus M, Woerle HJ, Struckmeier C, Katschinski M, Göke B, et al. GLP-1 Regulates Gastroduodenal Motility Involving Cholinergic Pathways. *J Neurogastroenterol Motil* (2009) 21(6):609–22. doi: 10.1111/j.1365-2982.2008.01246.x
20. Sathananthan M, Farrugia LP, Miles JM, Piccinini F, Dalla Man C, Zinsmeister AR, et al. Direct Effects of Exendin-(9,39) and GLP-1-(9,36) Amide on Insulin Action, B-Cell Function, and Glucose Metabolism in Nondiabetic Subjects. *Diabetes* (2013) 62:2752–6. doi: 10.2337/db13-0140
21. Meier JJ. GLP-1 Receptor Agonists for Individualized Treatment of Type 2 Diabetes Mellitus. *Nat Rev Endocrinol* (2012) 8:728–42. doi: 10.1038/nrendo.2012.140
22. Nauck MA, Quast DR, Wefers J, Meier JJ. GLP-1 Receptor Agonists in the Treatment of Type 2 Diabetes- State-of-the-Art. *Molec Metab* (2021) 46:101102. doi: 10.1016/j.molmet.2020.101102
23. Astrup A, Carraro R, Finer N, Harper A, Kunesova M, Lean MEJ, et al. Safety, Tolerability and Sustained Weight Loss Over 2 Years With the Once-Daily Human GLP-1 Analog, Liraglutide. *Int J Obes* (2012) 36:843–54. doi: 10.1038/ijo.2011.158
24. Ryan D, Acosta A. GLP-1 Receptor Agonists: Nonglycemic Clinical Effects in Weight Loss and Beyond. *Obesity* (2015) 23(6):1119–29. doi: 10.1002/oby.21107
25. Vilsbøll T, Krarup T, Madsbad S, Holst JJ. No Reactive Hypoglycaemia in Type 2 Diabetic Patients After Subcutaneous Administration of GLP-1 and Intravenous Glucose. *Diabet Med* (2001) 18(2):144–9. doi: 10.1046/j.1464-5491.2001.00424.x
26. Kendall DM, Riddle MC, Rosenstock J, Zhuang D, Kim DD, Fineman MS, et al. Effects of Exenatide (Exendin-4) on Glycemic Control Over 30 Weeks in Patients With Type 2 Diabetes Treated With Metformin and a Sulfonylurea. *Diabetes Care* (2005) 28(5):1083–91. doi: 10.2337/diacare.28.5.1083
27. Snider KE, Becker S, Boyajian L, Shyng S-L, MacMullen C, Hughes N, et al. Genotype and Phenotype Correlations in 417 Children With Congenital Hyperinsulinism. *J Clin Endocrinol Metab* (2013) 98(2):355–63. doi: 10.1210/jc.2012-2169
28. Lord K, De León DD. Hyperinsulinism in the Neonate. *Clin Perinatol* (2018) 45:61–74. doi: 10.1016/j.clp.2017.10.007
29. Shi Y, Avatapalle HB, Skae MS, Padidela R, Newbould M, Rigby L, et al. Increased Plasma Incretin Concentrations Identifies a Subset of Patients With Persistent Congenital Hyperinsulinism Without KATP Channel Gene Defects. *J Pediatr* (2015) 166:191–4. doi: 10.1016/j.jpeds.2014.09.019
30. De León DD, Li C, Delson MI, Matschisky FM, Stanley CA, Stoffers DA. Exendin-(9-39) Corrects Fasting Hypoglycemia in $SUR\text{-}1^{-/-}$ Mice by Lowering cAMP in Pancreatic Beta Cells and Inhibiting Insulin Secretion. *J Biol Chem* (2008) 283(38):25786–93. doi: 10.1074/jbc.M804372200
31. Fourtner SH, Stanley CA, Kelly A. Protein-Sensitive Hypoglycemia Without Leucine Sensitivity in Hyperinsulinism Cause by K(ATP) Channel Mutations. *J Pediatr* (2006) 149(1):47–52. doi: 10.1016/j.jpeds.2006.02.033
32. Calabria AC, Li C, Gallagher PR, Stanley CA, De Leon DD. GLP-1 Receptor Antagonist Exendin-(9-39) Elevates Fasting Blood Glucose Levels in Congenital Hyperinsulinism Owing to Inactivating Mutations in the ATP-Sensitive K+ Channel. *Diabetes* (2012) 62:2585–91. doi: 10.2337/db12-0166
33. Ng CM, Tang F, Seeholzer SH, Zou Y, De Leon DD. Population Pharmacokinetics of Exendin-(9-39) and Clinical Dose Selection in Patients

With Congenital Hyperinsulinism. *Br J Clin Pharmacol* (2018) 94:520–32. doi: 10.1111/bcp.13463
34. Craig C, De Leon D. Pharmacokinetics and Pharmacodynamics of Avexitide (Exendin 9-39) in Neonates and Infants With Congenital Hyperinsulinism. *Diabetes* (2021) 70, Supplement 1. doi: 10.2337/db21-79-LB
35. De Leon-Crutchlow D. Novel Therapies in the Treatment of Congenital Hyperinsulinism. *Hormone Res Paediatrics* (2021) 94:5. doi: 10.1159/000518849
36. FDA News. (2021). Available at: https://www.fdanews.com/articles/203878-eiger-gets-breakthrough-therapy-designation-for-congenital-hyperinsulinism-treatment?v=preview.
37. Papamargaritis D, le Roux CW. Do Gut Hormones Contribute to Weight Loss and Glycaemic Outcomes After Bariatric Surgery? *Nutrients* (2021) 13(3):762. doi: 10.3390/nu13030762
38. Calabria AC, Gallagher PR, Simmons R, Blinman T, De Leon DD. Postoperative Surveillance and Detection of Postprandial Hypoglycemia After Fundoplasty in Children. *J Pediatr* (2011) 159:597–601. doi: 10.1016/j.jpeds.2011.03.049
39. Palladino AA, Sayed S, Levitt Katz LE, Gallagher PR, De Leon DD. Increased Glucagon-Like-Peptide-1 Secretion and Post Prandial Hypoglycemia in Children After Nissen Fundoplication. *J Clin Endocrinol Metab* (2009) 94:39–44. doi: 10.1210/jc.2008-1263
40. De Leon DD. "Role of Incretin Hormones in Hyperinsulinemic Hypoglycemia". In: CA Stanley, DD De Leon, editors. *Monogenic Hyperinsulinemic Hypoglycemia Disorders*. Basel: Karger (2012). p. 79–86.
41. Yamoto M, Fukumoto K, Takahashi T, Miyake H, Nakaya K, Nomura A, et al. Risk Factors of Dumping Syndrome After Fundoplication for Gastroesphageal Reflux in Children. *Ped Surg Int* (2021) 37:183–9. doi: 10.1007/s00383-020-04783-0
42. Calabria AC, Charles L, Givler S, De Leon DD. Postprandial Hypoglycemia in Children After Gastric Surgery: Clinical Characterization and Pathophysiology. *Horm Res Paediatr* (2016) 85(2):140–6. doi: 10.1159/000442155
43. Miholic J, Ørskov C, Holst JJ, Kotzerke J, Meyer HJ. Emptying of the Gastric Substitute Glucagon-Like-Peptide-1 (GLP-1), and Reactive Hypoglycemia After Total Gastrectomy. *Dig Dis Sci* (1991) 36:1361–70. doi: 10.1007/BF01296800
44. McLaughlin T, Peck M, Holst J, Deacon C. Reversible Hyperinsulinemic Hypoglycemia After Gastric Bypass: A Consequence of Altered Nutrient Delivery. *J Clin Endocrinol Metab* (2010) 95:1851–5. doi: 10.1210/jc.2009-1628
45. Gebhard B, Holst JJ, Biegelmayer C, Miholic J. Postprandial GLP-1, Norepinephrine, and Reactive Hypoglycemia in Dumping Syndrome. *Dig Dis Sci* (2001) 46:1915–23. doi: 10.1023/A:1010635131228
46. Salehi M, Gastaldellli A, D'Alessio DA. Blockade of Glucagon-Like-Peptide 1 Receptor Corrects Postprandial Hypoglycemia After Gastric Bypass. *Gastroenterol* (2014) 146:669–80. doi: 10.1053/j.gastro.2013.11.044
47. Craig CM, Liu L, Nguyen T, Price C, Bingham C, McLaughlin TL, et al. Efficacy and Pharmacokinetics of Subcutaneous Exendin (9-39) in Patients With Post-Bariatric Hypoglycaemia. *Diabetes Obes Metab* (2018) 20(2):352–61. doi: 10.1111/dom.13078
48. Graig CM, Liu L-F, Deacon CF, Holst JJ, McLaughlin TL. Critical Role of GLP-1 in Symptomatic Post-Bariatric Hypoglycemia. *Diabetelogia* (2017) 60 (3):531–40.
49. Craig CM, Lawler HM, Lee CJE, Tan M, Belt Davis D, Tong J, et al. PREVENT: A Randomized, Placebo-Controlled Crossover Trial of Avexitide for Treatment of Postbariatric Hypoglycemia. *J Clin Endocrinol Metab* (2021) 106(8):3235–48. doi: 10.1210/clinem/dgab103
50. Tan M, Lamendola C, Luong R, McLaughlin T, Craig C. Safety, Efficacy and Pharmacokinetics of Repeat Subcutaneous Dosing of Avexitide (Exendin 9-39) for Treatment of Post-Bariatric Hypoglycemia. *Diabetes Obes Metab* (2020) 22(8):1406–16. doi: 10.1111/dom.14048

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Hypoglycaemia Metabolic Gene Panel Testing

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A large number of inborn errors of metabolism present with hypoglycemia. Impairment of glucose homeostasis may arise from different biochemical pathways involving insulin secretion, fatty acid oxidation, ketone bodies formation and degradation, glycogen metabolism, fructose and galactose metabolism, branched chain aminoacids and tyrosine metabolism, mitochondrial function and glycosylation proteins mechanisms. Historically, genetic analysis consisted of highly detailed molecular testing of nominated single genes. However, more recently, the genetic heterogeneity of these conditions imposed to perform extensive molecular testing within a useful timeframe *via* new generation sequencing technology. Indeed, the establishment of a rapid diagnosis drives specific nutritional and medical therapies. The biochemical and clinical phenotypes are critical to guide the molecular analysis toward those clusters of genes involved in specific pathways, and address data interpretation regarding the finding of possible disease-causing variants at first reported as variants of uncertain significance in known genes or the discovery of new disease genes. Also, the trio's analysis allows genetic counseling for recurrence risk in further pregnancies. Besides, this approach is allowing to expand the phenotypic characterization of a disease when pathogenic variants give raise to unexpected clinical pictures. Multidisciplinary input and collaboration are increasingly key for addressing the analysis and interpreting the significance of the genetic results, allowing rapidly their translation from bench to bedside.

Keywords: hypoglycemia, next generation sequencing, NGS, whole exome sequencing, whole genome sequencing

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1 INTRODUCTION

Hypoglycemia is associated with a large number of inborn errors of metabolism (IEM). The alteration of biochemical pathways involving carbohydrate, protein and lipid metabolism often leads to an impairment of glucose homeostasis (1–3). Although biochemical features of hypoglycemia are useful tools to undercover the underlying pathology, overlapping or unspecific features make arduous to reach a diagnosis at a short time. Indeed, metabolic diseases which can present with intermittent or persistent hypoglycemia include disorders of carbohydrate metabolism (glycogen storage diseases [GSDs], gluconeogenesis defects, hereditary fructose intolerance [HFI], galactosemia), hyperinsulinemic hypoglycemia (HI), fatty acid oxidation defects (FAODs), ketogenesis and ketolysis defects, mitochondrial DNA depletion syndromes, and some

aminoacidopathies (maple syrup urine disease, hepato-renal tyrosinemia [HT1], adenosine kinase deficiency). The establishment of a precise diagnosis is crucial to start specific nutritional and pharmacological therapies. Due to the multitude of genes associated with IEM, standard molecular approaches with Sanger sequencing for single genes would result expensive and time-consuming. In the last decade, next-generation sequencing (NGS) technologies have become essential tool for their rapid turnaround time and coverage in the field of metabolic diseases.

2 CLINICAL AND BIOCHEMICAL CHARACTERIZATION OF HYPOGLYCEMIA

According to current recommendations, hypoglycemia is defined as spontaneous symptomatic hypoglycemia and/or plasma glucose concentration <3.3 mmol/L (<60 mg/dL), or < 2.8 mmol/L (<50 mg/dL) after a provocative fasting test. In the differential diagnosis of hypoglycemia, it is important to evaluate the timing of hypoglycemia in relation to fasting state, eventual associated signs of visceral (hepatomegaly/hepatopathy, myopathy, cardiomyopathy) and neurologic involvement, and suggestive signs of hypopituitarism or adrenal insufficiency. Furthermore, a specific laboratory work-up needs to be performed at the time of hypoglycemia. Laboratory assays include routine analyses (plasma glucose, blood gas analysis, lactate, ammonia, uric acid, liver and muscle enzymes, free or non-esterified fatty acids [NEFA], triglycerides, blood and urinary ketones), endocrine (insulin, C-peptide, growth hormone, cortisol) and metabolic investigations (blood acylcarnitines, plasma aminoacids, urinary organic acids and transferrin isoforms) (1, 3).

The characterization in non-ketotic (or hypoketotic) and ketotic hypoglycemia (KH) distinguishes two major categories of disease: the first includes HI, hypopituitarism, GSD type I, FAOD and ketogenesis defects, the latter covers idiopatic ketotic hypoglycemia (IKH), single hormonal defects (cortisol deficiency, GH deficiency) and all the others metabolic diseases. Within non-ketotic hypoglycemias, suppressed NEFA are typical in HI and hypopituitarism.

3 IEM PRESENTING WITH HYPOGLYCEMIA IN CHILDHOOD

3.1 Disorders of Carbohydrate Metabolism

3.1.1 Hyperinsulinemic Hypoglycemia

The diagnosis of HI is defined by detectable plasma insulin level (>2–3 μU/ml) at the time of hypoglycemia or by signs of inappropriate excess of insulin, such as suppressed NEFA (<1.7 mM), hypoketonemia (<1.8 mM), a hyperglycemic response to i.m. glucagon (delta glucose>30 mg/dl in 30 min) and a high glucose demand (>10 mg/kg/min in neonates) (4–6).

Mutations in *ABCC8* and *KCNJ11* (encoding for the SUR1 and Kir6.2 subunits of the K_{ATP} channel, respectively) account for more than half of HI cases (7–11) and have been associated with two histological aspects of the endocrine pancreas. A diffuse form extended to all pancreatic β-cells, inherited as either autosomal recessive or dominant trait, and a focal form, which results from the combination of a paternally inherited germinal mutation and a somatic loss of heterozygosity of the maternal allele in a restricted group of β-cells (12). The two forms have different management and outcome, and hence differential diagnosis is a crucial point. Indeed, partial pancreatectomy is the elective procedure for focal HI and allows the complete recovery from hypoglycemia. Therefore, finding a genotype suggestive for a focal form determines the subsequent diagnostic pathway through 18F-DOPA PET/CT (13).

Beyond *ABCC8* and *KCNJ11*, mutations in pancreatic genes involved in fatty acid oxidation, energy and aminoacid metabolism and transcription factors give rise to diffuse forms of HI: *GCK*, *GLUD1*, *HADH*, *HNA4a*, *HNF1a*, *SLC16A1*, *UCP2*, *HK1*, *INSR* (13, 14). Except GLUD1-HI, in which hyperammonemia is a characteristic finding, in all other diffuse forms there is no recognized biomarker, and molecular analysis is the only tool to make a specific diagnosis.

Diffuse HI is treated with medical therapy (diazoxide, octreotide), and only in case of unresponsiveness, a near-total pancreatectomy is required. However, this procedure is often associated with increased risk of diabetes and exocrine pancreatic failure (15–18), and does not guarantee the remission of hypoglycemia (17). For these reasons, in severe unresponsive forms of GCK-HI, a therapeutic approach with ketogenic diet was recently successfully proposed as elective treatment, because patients recovered from epilepsy, intellectual disabilities and symptoms of recurrent hypoglycemia (19).

Furthermore, specific mutations p.R63W and LRG_483t1: c.427-1G>A in *HNF4α* cause HI associated to hepatomegaly and renal Fanconi syndrome, a phenotype similar to Fanconi-Bickel syndrome (due to inactivating mutations of *SLC2A2* resulting in nonfunctional glucose transporter 2, GLUT2). This *HNF4α* mutations might decrease the expression of *SLC2A2* in both liver and kidney, and are responsive to diazoxide therapy, unlike the Fanconi-Bickel syndrome (20, 21).

HI also occurs in some congenital disorders of glycosylation, such as PMM2-CDG (22), PMI-CDG (23), ALG6-CDG (24), ALG3-CDG (25), and PGM1-CDG (26, 27).

PMI-CDG and PGM1-CDG are treatable disorders with mannose and galactose therapy, respectively. Therefore, the genetic characterization leads the therapeutic choices.

Several genetic syndromes have been particularly associated with HI or with KH. Syndromic hypoglycemias caused by mutations in known genes are listed in **Table 1**. However, syndromic conditions due to chromosomal aberrations (e.g. Turner syndrome, Trisomy 13, Trisomy 21) or genetic deletions (e.g. Usher syndrome caused by contiguous gene deletion including *USH1C* and *ABCC8*; 16p11.2 deletion, 22q11.2 deletion, 9p deletion) or epigenetic alterations (e.g. Beckwith-Wiedemann, Silver-Russel, Prader Willi syndromes) or undiagnosed dysmorfisms have been reported to be associated with hypoglycemia (13, 28).

TABLE 1 | Genes associated to Hypoglycemia.

GENE	Disease	Inheritance	OMIM
Hyperinsulinemic Hypoglycemia			
ABCC8	Hyperinsulinemic hypoglycemia, familial, 1	AD, AR	# 256450
KCNJ11	Hyperinsulinemic hypoglycemia, familial, 2	AD, AR	# 601820
GLUD1	Hyperinsulinism-hyperammonemia syndrome	AD	# 606762
GCK	Hyperinsulinemic hypoglycemia, familial, 3	AD	# 602485
HADHSC	Hyperinsulinemic hypoglycemia, familial, 4	AR	# 609975
HNF4A	MODY, type I	AD	# 125850
SLC16A1	Hyperinsulinemic hypoglycemia, familial, 7	AD	# 610021
UCP2	Obesity, susceptibility to, BMIQ4	AD	# 601693
HNF1A	MODY, type III	AD	# 600496
INSR	Hyperinsulinemic hypoglycemia, familial, 5	AD	# 609968
HK1	Hexokinase 1	AR	*142600
Glycogen storage diseases			
G6PC1	Glycogen storage disease Ia	AR	# 232200
SLC37A4	Glycogen storage disease Ib	AR	# 232220
AGL	Glycogen storage disease III	AR	# 232400
GBE1	Glycogen storage disease IV	AR	# 232500
PYGL	Glycogen storage disease VI	AR	# 232700
PHKA2	Glycogen storage disease IXa	XLR	# 306000
PHKB	Glycogen storage disease IXb	AR	# 261750
PHKG2	Glycogen storage disease IXc	AR	# 613027
SLC2A2	Fanconi-Bickel syndrome	AR	# 227810
GYS2	Glycogen storage disease 0, liver	AR	# 240600
Gluconeogenesis defects			
FBP1	Fructose-1,6-bisphosphatase deficiency	AR	# 229700
PCK1	Phosphoenolpyruvate carboxykinase deficiency	AR	# 261680
Hereditary fructose intolerance			
ALDOB	Hereditary fructose intolerance	AR	# 229600
Galactosemia			
GALT	Galactosemia 1	AR	# 230400
GALE	Galactose epimerase deficiency	AR	# 230350
Congenital disorders of glycosylation			
PMM2	Congenital disorder of glycosylation, type Ia	AR	# 212065
MPI	Congenital disorder of glycosylation, type Ib	AR	# 602579
ALG6	Congenital disorder of glycosylation, type Ic	AR	# 603147
ALG3	Congenital disorder of glycosylation, type Id	AR	# 601110
PGM1	Congenital disorder of glycosylation, type It	AR	# 614921
β-oxidation defects			
SLC22A5	Carnitine deficiency, systemic primary	AR	# 212140
CPT1A	CPT deficiency, hepatic, type IA	AR	# 255120
SLC25A20	Carnitine-acylcarnitine translocase deficiency	AR	# 212138
CPT2	CPT II deficiency	AR	# 600649
ACADVL	VLCAD deficiency	AR	# 201475
HADHA	LCHAD deficiency	AR	# 609016
HADHB	Trifunctional protein deficiency	AR	# 609015
ACADM	Acyl-CoA dehydrogenase, medium chain, deficiency	AR	# 201450
ACADS	Acyl-CoA dehydrogenase, short-chain, deficiency	AR	# 201470
HADH	3-hydroxyacyl-CoA dehydrogenase deficiency	AR	# 231530
ETFA	Glutaric acidemia IIA	AR	# 231680
ETFB	Glutaric acidemia IIB	AR	# 231680

(Continued)

TABLE 1 | Continued

GENE	Disease	Inheritance	OMIM
ETFDH	Glutaric acidemia IIC	AR	# 231680
Ketogenesis defects			
HMGCS2	HMG-CoA synthase-2 deficiency	AR	# 605911
HMGCL	HMG-CoA lyase deficiency	AR	# 246450
Ketolysis defects			
ACAT1	Beta-ketothiolase deficiency	AR	# 203750
SLC16A1	Monocarboxylate transporter 1 deficiency	AD, AR	# 616095
Organic acidemia			
MMUT	Methylmalonic aciduria, mut (0) type	AR	# 251000
MMAA	Methylmalonic acidemia, cblA	AR	# 251100
MMAB	Methylmalonic acidemia, cblB	AR	# 251110
PCCA	Propionic acidemia	AR	# 606054
PCCB	Propionic acidemia	AR	# 606054
IVD	Isovaleric acidemia	AR	# 243500
Maple syrup urine disease			
BCKDHA	Maple syrup urine disease, type Ia	AR	# 248600
BCKDHB	Maple syrup urine disease, type Ib	AR	# 248600
DBT	Maple syrup urine disease, type II	AR	# 248600
DLD	Maple syrup urine disease, type III	AR	# 246900
Tyrosinemia type 1			
FAH	Hepato-renal tyrosinemia (Tyrosinemia, type I)	AR	# 276700
Adenosine kinase deficiency			
ADK	Adenosine kinase deficiency	AR	# 614300
Mitochondrial DNA depletion syndrome			
MPV17	Mitochondrial DNA depletion syndrome 6 (hepatocerebral type)	AR	# 256810
DGUOK	Mitochondrial DNA depletion syndrome 3 (hepatocerebral type)	AR	# 251880
SUCLG1	Mitochondrial DNA depletion syndrome 9	AR	# 245400
TWNK	Mitochondrial DNA depletion syndrome 7 (hepatocerebral type)	AR	# 271245
TFAM	Mitochondrial DNA depletion syndrome 15 (hepatocerebral type)	AR	# 617156
POLG	Mitochondrial DNA depletion syndrome 4A	AR	# 203700
Other genes			
APPL1	Maturity-onset diabetes of the young, type 14	AD	# 616511
BLK	Maturity-onset diabetes of the young, type 11	AD	# 613375
CEL	Maturity-onset diabetes of the young, type VIII	AD	# 609812
GK	Glycerol kinase deficiency	XLR	# 307030
HNF1B	Type 2 diabetes mellitus	AD	# 125853
IGF1R	Insulin-like growth factor I, resistance to	AD, AR	# 270450
INS	Hyperproinsulinemia	AD	# 616214
KLF11	Maturity-onset diabetes of the young, type VII	AD	# 610508
MAFA	Insulinomatosis and diabetes mellitus	AD	# 147630
NEUROD1	Diabetes mellitus, type 2 susceptibility to	AD	# 125853
PAX4	Diabetes mellitus, type 2 susceptibility to	AD	# 125853
PDX1	Diabetes mellitus, type 2 susceptibility to	AD	# 125853
SLC5A1	Glucose/galactose malabsorption	AR	# 182380
SLC25A13	Citrullinemia type II	AR	# 603859
ACAD9	ACAD9 deficiency	AR	# 611103
Syndromic Hypoglycemia			
CDKN1C	Beckwith-Wiedemann syndrome	AR	# 130650
CACNA1C	Timothy syndrome	AD	# 601005
NSD1	Sotos syndrome 1	AD	# 117550
GPC3	Simpson-Golabi-Behmel syndrome	XLR	# 312870
HRAS	Costello syndrome	AD	# 218040
DIS3L2	Perlman syndrome	AR	# 267000

(Continued)

TABLE 1 | Continued

GENE	Disease	Inheritance	OMIM
KMT2D	Kabuki syndrome 1	AD	# 147920
KDM6A	Kabuki syndrome 2	XLD	# 300867
GHR	Laron syndrome	AR	# 262500
PHOX2B	Ondine (central hypoventilation syndrome)	AD	# 209880
TRMT10A	Microcephaly, short stature, and impaired glucose metabolism 1	AR	# 616033
ARID1B	Coffin-Siris syndrome 1	AD	# 135900
CHD7	CHARGE syndrome	AD	# 214800
CREBBP	Rubinstein-Taybi syndrome 1	AD	# 180849
EP300	Rubinstein-Taybi syndrome 2	AD	# 613684
JAG1	Alagille syndrome	AD	# 118450
RNF125	Tenorio syndrome	AD	# 610432
AKT2	Hypoinsulinemic hypoglycemia with hemihypertrophy	AD	# 240900
PIK3R2	Megalencephaly-polymicrogyria syndrome	AD	# 603387
AKT3	Megalencephaly-polymicrogyria syndrome	AD	# 615937
PIK3CA	Phosphatidylinositol 3-kinase, catalytic, alpha		*171834
CCND2	Cyclin D2		*123833
APC2	Cortical dysplasia, complex, with other brain malformations	AR	# 618677
PLAGL1	Silver-Russell syndrome 4	AD	# 618907
CACNA1D	Primary aldosteronism, seizures, and neurologic abnormalities	AD	# 615474
New Genes			
NCOR1	Nuclear Receptor corepressor 1		* 600849
IGF2BP1	Insulin like growth factor 2 mRNA binding protein 1		* 608288
SLC5A2	Renal glucosuria	AD, AR	# 233100
NEK11	NIMA related kinase 2		* 604043
FOXA2	Hepatocyte nuclear factor 3, beta		* 600288
EIF2S3	MEHMO syndrome	XLR	# 300148
DNAJC3	Neuroendocrine developmental disorder with insulin dysregulation	AR	# 616192

#OMIM phenotype description, molecular basis known.
**OMIM gene description.*

3.1.2 Glycogen Storage Diseases

GSDs are IEM involving synthesis and degradation of glycogen, resulting in a failure to convert glycogen into energy, and in a glycogen accumulation in multiple organs. Glycogen is a branched polymer of glucose molecules. After a meal, plasma glucose increases and stimulates the storage of excess glucose in form of cytoplasmic glycogen in many tissues as liver, skeletal muscle, heart and kidney. Hepatic GSDs present with hypoglycemia, particularly the major types GSD Ia, Ib and III. Dietary treatment is the cornerstone of management aiming at maintenance of euglycaemia, prevention of secondary metabolic perturbations, and long-term complications affecting multiple organs, such as liver (hepatocellular adenomas and carcinomas), heart (cardiomyopathy), muscle (myopathy), kidneys (proteinuria, renal insufficiency, stones), and bone (osteopenia, osteoporosis). According to GSD type and age, patients are treated with hyperglucidic diet with frequent feeds, continuous nocturnal gastric drip feeding or late evening meal supplemented with uncooked cornstarch, or restriction of mono- and disaccharides, or hyperproteic diet (29).

GSD type I is a rare disease of variable clinical severity that primarily affects the liver and kidney. It is caused by deficient activity of the glucose 6-phosphatase enzyme (GSD Ia) or a deficiency in the microsomal transport proteins for glucose 6-phosphate (GSD Ib), resulting in excessive accumulation of glycogen and fat in the liver, kidney, and intestinal mucosa. Patients have a wide spectrum of clinical and biochemical manifestations, including hepatomegaly, growth retardation, hypoketotic hypoglycemia, hyperlactatemia, metabolic acidosis, hyperuricemia and hyperlipidemia. Since both glycogenolysis and gluconeogenesis are affected, individuals with GSD type Ia typically manifest hypoglycemia in infancy when the interval between feedings is extended to 2-3 hours. Rate of complications and disease severity are variable. In addition, patients with GSD type Ib manifest neutropenia and neutrophil dysfunction, recurrent infections and inflammatory bowel disease (30). In the first two years of life, the phenotype of the two forms is undistinguishable until neutropenia appears. Patients are treated with frequent feeds of hyperglucidic, hypolipidic diet, added with maltodextrin and cornstarch, and with nocturnal enteral feeding in the first year of life. Recently, in GSD type Ib a novel treatment with empagliflozin appeared effective in controlling neutrophil dysfunction and inflammatory bowel disease (31).

GSD type III is caused by recessive mutations in the *AGL* gene with consequent deficiency of the glycogen debranching enzyme.

Patients manifest hepatomegaly, growth retardation, KH, hyperlipidemia and elevated liver enzymes. Phenotypically, patients can be further classified into having GSD type IIIa (85%), with involvement of the liver, heart, and skeletal muscle, or GSDIIIb (15%), in which only the liver is affected (32). Since gluconeogenesis is unaffected, patients with GSD type IIIb are commonly treated with a high protein diet, and cornstarch if necessary. GSD type IIIa with cardiomyopathy is an elective indication for ketogenic diet, which completely reverses the cardiac hypertrophy (33–36).

The other forms of hepatic GSDs type IV, VI, IXa, IXb, IXc typically present with hepatomegaly, elevated liver enzymes, dyslipidemia, growth retardation, renal tubular dysfunction and can present with KH. Liver GSDs take overlapping symptoms and can be clinically indistinguishable.

GSD type IV is caused by recessive mutations in the *GBE1* gene, which leads to 1,4-α-glucan-branching enzyme deficiency. GBE deficiency involves the liver, the neuromuscular system and the heart. In the classical (progressive) hepatic phenotype, infants develop hepatomegaly, hypoglycemia, hypotonia, and developmental delay during the first months of life, with rapid progression to portal hypertension, ascites and liver cirrhosis between the second and fourth year of life. A nonprogressing form with exclusively liver involvement has been reported in a few cases. Neuromuscular symptoms may appear from fetal to adult age. The most severe form starts *in utero* with decreased fetal movements, arthrogryposis, hypoplastic lungs, and may cause perinatal death. Patients are treated with hyperglucidic diet plus cornstarch, nocturnal enteral feeding, protein enrichment, and in the more severe form, with liver transplantation (37).

GSD type VI presents as a relatively mild disorder in infancy and childhood (38–40). It is caused by recessive mutations or deletions of the *PYGL* gene (deficiency of liver phosphorylase) (41).

GSD type IX is the most frequent hepatic GSD resulting from a deficient liver phosphorylase kinase (PhK) system. GSD type IXa (*PHKA2* mutations) is the most common subtype of liver PhK deficiency, accounting for 75% of GSD type IX, with an X-linked inheritance. Patients usually manifest hepatomegaly, hepatopathy, hypoglycemia and renal tubulopathy with a milder or benign courses. Conversely, patients with GSD type IXc (*PHKG2* mutations) have more severe clinical features such as mild gross motor delays, hypoglycemia, liver fibrosis and cirrhosis in childhood (42). Patients are treated with high protein diet, and cornstarch if needed.

Fanconi-Bickel syndrome (also known as GSD type XI) is caused by mutations in the GLUT2 (*SLC2A2*) gene. It is characterized by glycogen accumulation in liver and kidneys, with fasting hypoglycemia, hepatomegaly, tubular nephropathy (glucosuria, proteinuria, phosphaturia, bicarbonate wasting, and aminoaciduria), rickets, failure to thrive and short stature. The phenotypic variability ranges from mild presentation to diabetes mellitus (43, 44). Patients are treated with hyperglucidic diet with low content of galactose.

In the hepatic GSD type 0, caused by mutations in *GYS2*, glycogen synthesis is impaired. As glucose cannot be converted to glycogen, patients manifest fasting hypoglycemia and postprandial hyperglycemia. Postprandial hyperlactatemia also develops for the conversion of meal-derived carbohydrates to lactate. Fasting ketotic hypoglycemia usually manifests in late infancy when overnight feedings are stopped. Since gluconeogenesis and fatty acid oxidation are unaffected, in general the cognitive function is normal. Short stature and osteopenia are common features (45–48). The disease is underdiagnosed. Patients are treated with frequent feeds of hyperglucidic and hyperproteic diet.

3.1.3 Gluconeogenesis Defects

Gluconeogenesis plays an essential role in glucose homeostasis. Through this pathway, amino acids, lactate, glycerol, and other non-carbohydrate substrates are converted into glucose to meet energy demands under prolonged starvation, infections or metabolic stress (49).

Fructose-1,6-phosphatase (*FBP1*) deficiency manifests in the neonatal period or later on with KH, iperlactatemia, metabolic acidosis, hyperuricemia, hepatomegaly during decompensations. Patients present hyperalaninemia and glucagon-unresponsiveness. Elevated levels of glycerol 3-phosphate can be found in urine organic acid analysis (50). Alterations of consciousness can progress into coma. Episodes are typically triggered by fasting, infections, or ingestion of large amounts of fructose. Patients need to avoid fasting, they are treated with frequent feeds, often with added cornstarch. Tolerance to fasting improves with age (51). The presence of urinary glycerol 3-phosphate puts the disease in differential diagnosis with the Glycerol kinase deficiency, an X-linked recessive disorder characterized by hyperglycerolaemia and glyceroluria. Indeed, children affected by the juvenile form of the latter condition may present with Reye-like episodes of vomiting, metabolic acidosis and KH with progressive unconsciousness during intercurrent illnesses, and "pseudohypertriglyceridaemia" as a result of a raised plasma glycerol (52).

Cytosolic Phosphoenolpyruvate carboxykinase (*PEPCK, PCK1*) deficiency begins in neonatal age or after a few months. Beyond the phenotypic alterations described for FBP1 deficiency, patients display mostly progressive neurologic involvement with hypotonia, developmental delay, epilepsy, spasticity, microcephaly and multiorgan damage with hepatomegaly, hepatocellular dysfunction, cardiomyopathy, muscular weakness, renal tubular acidosis, and failure to thrive. The clinical picture may also mimic Reye syndrome (53, 54). Urine organic acids profile shows low or absent ketonuria with presence of fumarate, adipate, succinate, 2-ketoglutarate and glutarate, sometimes ethylmalonate, and in some patients a profile similar to those seen in defects of ketogenesis has been reported (55). Treatment is based on high carbohydrate diet plus cornstarch, and avoidance of fasting.

3.1.4 Hereditary Fructose Intolerance

Hereditary fructose intolerance is an autosomal-recessive disorder caused by deficiency of aldolase B. Upon introduction of fructose-containing foods patients manifest abdominal pain, nausea, recurrent vomiting, hypoglycemia, lactic acidemia, hypophosphatemia, hyperuricemia in case of acute fructose

intoxication. Parenteral intravenously administration of fructose, sorbitol, or sucrose may be life threatening for severe hypoglycemia and acute hepato-renal failure, and must be rigorously avoided (56). ATP depletion with toxic effect on glycogenolysis and gluconeogenesis is responsible of hypoglycemia. Many individuals with HFI exhibit a self-imposed aversion to fructose-containing foods, sufficiently to prevent an acute intoxication. However, prolonged fructose intake leads to poor feeding, vomiting, failure to thrive, hepatomegaly, liver and renal tubular dysfunction that might lead to irreversible liver and kidney damage (57, 58). Upon dietary restriction of fructose, symptoms resolve and normal growth and development is achieved. Therefore, individuals with HFI need to be treated with a fructose, sorbitol, sucrose-restricted diet. The disease can be misdiagnosed because some individuals can only manifest fruit aversion. However, the ingestion of certain medicinal formulations containing fructose or analogue sugars can cause severe hypoglycemia and acute hepato-renal failure (58).

3.1.5 Galactosemia

Infants with galactosemia typically present within the first few days of life with liver failure with coagulopathy, jaundice, hepatomegaly, hypoglycemia, seizures, cerebral edema after exposure to dietary galactose in the form of breastmilk or standard infant formulas. Additional findings may include poor weight gain, lethargia, renal failure, cataracts, vitreous hemorrhage and Escherichia coli sepsis. The disease with presentation of acute liver failure and hypoglycemia is caused by recessive mutations of galactose-1-phosphate uridyltransferase (*GALT*) and uridine diphosphate-galactose 4-epimerase (*GALE*). Long-term outcomes are oro-motor dyspraxia, intellectual disabilities, tremors and ataxia, ovarian dysfunction, osteoporosis. The therapy consists in low galactose/lactose diet (59).

3.1.6 Congenital Disorders of Glycosylation

Congenital disorders of glycosylation (CDGs) are complex rare diseases involved in protein glycosylation with functional consequences in protein folding, endocrine function, immune response, coagulation, cell interaction and signal transduction. A characteristic marker is altered glycosylation of transferrin visible at isoelectrofocusing of serum transferrin.

Phosphomannomutase 2 (PMM2)-CDG, Glucosyltransferase 1 (ALG6)-CDG and Mannosyltransferase 6 (ALG3)-CDG are complex disorders with multiorgan involvement and can present with HI.

PMM2-CDG is characterized by a neurological picture of internal strabismus, psychomotor disability, ataxia, cerebellar hypoplasia, epilepsy and classical features of inverted nipples and abnormal subcutaneous adipose tissue distribution. Nearly all other organs can be involved (22). ALG6-CDG presents with psychomotor disability, neurological symptoms, behavioural problems, skeletal abnormalities, and often protein-losing enteropathy (24). ALG3-CDG leads to severe neurological and skeleton involvement (25).

Phosphomannoisomerase (PMI)-CGD is a complex non neurologic syndrome characterized by protein-losing enteropathy, hepatopathy/liver failure, coagulopathy, HI and normal development. PMI catalyzes the conversion of fructose-6-P in mannose-6-P. HI patients are responsive to diazoxide. Therapy with mannose (which can be converted to mannose-6-P by hesokinase enzyme) restores intestinal and hepatic function, coagulation, hypoglycemia and the isoelectrofocusing of serum transferrin (23).

Phosphoglucomutase 1 (PGM1)-CDG has a complex phenotype characterized by hepatopathy, myopathy, exercise-induced rhabdomyolysis, cardiomyopathy, bifid uvula, growth retardation, coagulation and endocrine alterations. It is also called GSD type XIV, because patients show a combination of fasting KH, with post-prandial HI. Since PGM1 catalyzes the transfer of phosphate between glucose-1-P and glucose-6-P, the proposed mechanisms are impaired carbohydrate metabolism of the glycogen pathway for fasting KH, and a lower glucose threshold for insulin secretion caused by the increased glucose-6-P for post-prandial HI. Therapy with oral galactose improves hypoglycemia, endocrine abnormalities and coagulation as well as transferrin glycosylation pattern (26, 27).

3.2 Disorders of Lipid Metabolism

3.2.1 β-Oxidation Defects

The oxidation of fatty acids in mithocondria plays an important role in energy production. During late stages of fasting, fatty acids are released from adipose tissue triglyceride stores. Their oxidation spares glucose consumption and the need to use proteins to form glucose. Furthermore, the oxidation of fatty acids by the liver provides energy for gluconeogenesis and ureagenesis. Long-chain fatty acids are used by the heart and skeletal muscle during sustained exercise. In the liver, they are converted in ketone bodies, which serve as a fuel for brain, and thus further reduce the need for glucose utilization. Therefore, FAODs are characterized by fasting or stress induced hypoketotic hypoglycemia with increased NEFA, and presents with three major signs of hepatic, skeletal muscle and cardiac involvement: raised liver and/or muscle enzymes (hepatopathy/rabdomyolysis) with or without hypoglycemia, cardiomyopathy and arrhythmias. Specific biomarkers are abnormal acylcarnitines and/or urinary organic acids. The foundation of therapy is to prevent metabolic decompensations, avoiding fasting stress.

Defects in carnitine cycle, in very long chain-, long chain-, medium chain-, short chain dehydrogenases and in the electron transfer pathway cause different forms of FAODs, which require different nutritional (low-fat diet and medium chain triglycerides administration in some long chain FAODs) and therapeutic approaches or no approach. In addition, a single defect may have a variety of clinical manifestations even within the same family, as the case of multiple acyl-CoA dehydrogenase deficiencies (MADD), which ranges from hypoketotic hypoglycaemia, metabolic acidosis, cardiomyopathy to leukodystrophy, neurodevelopmental delay and myopathy (60). Genetic analysis has a pivotal role for diagnosis and prognosis establishment, and allows to personalize the treatment.

3.2.2 Ketogenesis Defects

During fasting, ketone bodies are important fuels for many tissues, such as brain, heart and skeletal muscle. Disorders of ketone bodies

formation present either in the first few days of life or later in childhood, during infections, prolonged fasting or other metabolic stress. During decompensation patients present encephalopathy with vomiting and a reduced level of consciousness, and often hepatomegaly. The biochemical features, hypoketotic hypoglycemia with or without hyperammonemia, resemble those of FAODs, but normal acylcarnitine profile is present. Recessive mutations of HMG-CoA synthase (*HMGCS2*) and HMG-CoA lyase (*HMGCL*) deficiency are responsible of ketogenesis defects (61–65). Urine organic acid profiles during decompensation are usually dominated by secondary products of fatty acid oxidation, with a characteristic 4-hydroxy-6-methyl-2-pyrone (4-HMP) in HMG-CoA synthase deficiency. However, molecular analysis is essential for diagnosis (63). Avoidance of fasting and a high carbohydrate intake need to be maintained to prevent decompensations (61). HMG-CoA lyase deficiency is a life-threatening metabolic intoxication with a presentation mimicking a Reye syndrome including recurrent vomiting, severe non-ketotic hypoglycemia, metabolic acidosis, hyperammonemia, hepatomegaly, seizures, and coma triggered by a catabolic state such as infections or low dietary intake. Generally, the clinical onset is within the first year of age. However, epilepsy, lethargy, hepatomegaly, anemia and eating difficulties have been reported in older children (64, 65). Urine organic acids analysis shows a typical profile including high levels of 3-Hydroxy-3-MethylGlutaric, 3-MethylGlutaric, 3-MethylGlutaconic and 3-HydroxyIsovaleric acids, and an acylcarnitine profile revealing a high level of 3-hydroxy-isovalerylcarnitine with a decreased free carnitine concentration (66). The treatment is based on a protein- and fat-restricted diet. L-carnitine supplementation is recommended. The long-term outcome in older children is characterized by neurological complications such as epilepsy, muscular hypotonia and tremor associated with white matter lesions in the brain MRI (65).

3.2.3 Ketolysis Defects

Ketolysis defects involve ketone utilization in extrahepatic tissues. The hallmark of decompensation is severe ketoacidosis. Two disorders may also present with hypoglycemia.

Beta-ketothiolase deficiency is an IEM that affects isoleucine catabolism and ketone body metabolism. Patients manifest intermittent ketoacidotic crises and hypoglycemia under catabolic stresses. Most patients developed their first crises between the ages of 6 months and 3 years. Neurological outcome, such as particularly extrapyramidal signs can occur, even in patients without any apparent decompensation (67). A characteristic increase in urinary 2-methyl-3-hydroxybutyrate and tiglylglycine, and a raise of C4OH levels at acylcarnitine profile are typical metabolic biomarkers (68).

Monocarboxylase transporter 1 (MCT1) mediates transport of pyruvate, lactate and ketone bodies across cell membranes. Patients with heterozygous or homozygous inhibiting mutations in *SLC16A1* present with moderate or profound ketosis and sometimes hypoglycemia during fasting or infections, within the first years of life (69, 70). In some patients migraine, exercise intolerance, developmental delay, microcephaly and abnormal MRI of the brain have been reported (71).

3.3 Disorders of Aminoacid Metabolism

3.3.1 Organic Acidemias and Maple Syrup Urine Disease

In organic acidemias (methylmalonic, propionic and isovaleric acidemia) and maple syrup urine disease, the enzymatic deficiency (methylmalonyl-CoA mutase, propionyl-CoA carboxylase, isovaleryl-CoA dehydrogenase, and the branched chain ketoacids dehydrogenase complex, respectively) is responsible of a cellular intoxication with energy deprivation causing poor feeding, vomiting, seizures, respiratory distress, metabolic acidosis, ketonuria, increased serum ammonia, lethargy, encephalopathy progressing to coma. Inhibition of gluconeogenesis can lead to hypoglycaemia. Urinary organic acids, acylcarnitines and plasma aminoacids are essential diagnostic biomarkers. The conditions are typically detected on metabolic newborn screening and are treated by a nutritional regimen with limited intake of intact proteins (which contain branched chain aminoacids), while providing adequate branched chain aminoacids-free exogenous proteins *via* medical food, with the aim to reduce catabolism, promote anabolism and preserve normal growth and development (72–74).

3.3.2 Hepato-Renal Tyrosinemia

Hepatorenal Tyrosinaemia or Tyrosinaemia Type 1 (HT1) is an autosomal recessive IEM caused by deficiency of the enzyme fumarylacetocetase in the pathway of tyrosine catabolism. This leads to the accumulation of tyrosine and toxic upstream intermediates such as succinylacetone, visible in urinary organic acids. Without treatment, HT1 patients suffer from liver failure and/or renal tubular dysfunction, and hepatocellular carcinoma (HCC) is a common long-term complication. HI is relatively common in untreated HT1. Pharmacological treatment consists of nitisinone along with a protein-restricted diet supplemented with aminoacid-mixtures free of tyrosine (75).

3.3.3 Adenosine Kinase Deficiency

Adenosine kinase deficiency is a rare recessive disorder of methionine and adenosine metabolism with a severe clinical phenotype comprising mainly neurological and hepatic impairment and dysmorphisms. There is phenotypic variability from neurological to a multi-organ involvement, with hepatic steatosis to severe neonatal liver dysfunction, hypotonia, developmental delay and dysmorphisms. Many patients manifest epilepsy and HI, not always responsive to diazoxide. Biochemical markers are intermittent hypermethioninemia, increased plasma S-adenosylmethionine (SAM), S-adenosylhomocysteine (SAH) and adenosine, intermittent dicarboxylic aciduria with normal acylcarnitines. A methionine restricted diet improved clinical and biochemical signs in some patients (76).

3.4 Mitochondrial DNA Depletion Syndrome

Defects in any protein involved in mtDNA maintenance causing quantitative and qualitative defects are classified as

mitochondrial DNA depletion syndrome (MDDS). Three clinical phenotypes have been described: hepatocerebral, encephalomyopathic and myopathic.

Mutations in deoxyguanosine kinase (*DGUOK*), mitochondrial inner membrane protein *MPV17*, polymerase catalytic subunit (*POLG*), succinate-CoA ligase GDP/ADP-forming subunit alpha (*SUCLG1*), twinkle MtDNA helicase (*TWNK*) and transcription factors A (*TFAM*) have been associated with hepatocerebral MDDS, with acute liver failure in infancy and hypoglycemia. *DGUOK* deficiency is one of the most frequent causes of hepatocerebral dysfunction (77). Recessive mutations of *POLG*, the main gene of mtDNA replication, are associated with a phenotype ranging from infantile hepatopathic poliodystrophy (Alpers-Huttenlocher syndrome) to sensory-ataxia neuropathy with dysarthria and ophthalmoplegia (SANDO), and spinocerebellar ataxia-epilepsy syndrome (SCAE) (78, 79). The Alpers-Huttenlocher syndrome is characterized by hepatic involvement with jaundice, cholestasis, hepatomegaly, elevated transaminases, evolving into liver failure with hypoglycemia associated with progressive neurologic symptoms and refractory epilepsy (79). Patients diagnosed with *DGUOK* mutations present with low birth weight and in a few weeks manifest neurological signs of rotatory nystagmus, hypotonia, and developmental delay, associated with hypoglycemia, raise of lactate and plasma alanine (80). *SUCLG1* deficiency has a characteristic methylmalonic aciduria at urinary organic acids (81).

4 ROLE OF GENETICS

In hypoglycemia-associated IEM, a rapid diagnosis is essential to establish appropriate and specific dietetic and pharmacological therapies, which are crucial for the short and long-term prognosis.

Although clinical characteristics of patients and laboratory signs may address the diagnosis, there are often overlapping phenotypes that induce uncertainty as well as mutations in multiple candidate genes can give rise to the same phenotype in the field of a specific disorder (e.g. HI, GSDs, MDDS). For these reasons, Sanger sequencing for single genes at a time is not applicable because time- and cost consuming (1, 82). In these conditions in which a genetic diagnosis allows to settle the more appropriate treatment, a rapid turnaround time is particularly significant. Genetic analysis influences decision making even in acute inpatient setting, as the case of HI in which the decision to candidate the patient to partial pancreatectomy depends on the finding of a recessive paternally inherited mutation in *ABCC8* or *KCNJ11*, which is suggestive of a focal form (13, 15). Furthermore, patients with KH without metabolic and hormonal biomarkers are often classified within idiopatic ketotic hypoglycemia (IKH). IKH has been mostly considered as a non-pathological condition of children with a fasting tolerance at the lower tail of the Gaussian normal distribution until school age (83). However, some children continue to present IKH until adulthood. Pathological KH has been defined as recurrent episodes with blood glucose < 70 mg/dl (3.9 mmol/L) and betahydroxybutyrate ≥ 1.0 mmol/L, without any trigger of fasting or infections. Pathological KH may be due to underlying diseases, e.g. GSDs, defective MCT and genetic syndromes or to novel diseases that can be identified by whole exome sequencing (WES). The treatment consists in prevention of hypoglycemia and protein deficiency by adequate supplementation of carbohydrates and proteins, with uncooked cornstarch, or continuous tube feeding by night. Failure to settle a proper diagnosis of IKH may lead to undertreatment (71). Indeed, the ketotic forms of GSDs are underrecognized because endocrinological and metabolic parameters are unremarkable during investigations for hypoglycemia (84). Brown et al. found variants in genes causing GSDs (including type 0, VI, IXa, IXb, IXc) in 12% of IKH patients without hepatomegaly, by performing Sanger sequencing on five genes (*GYS2*, *PYGL*, *PHKA2*, *PHKB*, and *PHKG2*) (85). This finding was unexpected, because GSDs are usually suspected in case of hepatomegaly and raise of transaminases. This finding led other physicians to perform NGS in a patient with frequent IKH without hepatomegaly or elevated liver enzyme levels, unravelling a rare variant in the *PHKA2* gene. In those cases, KH patients should be treated with hyperproteic diet, similarly to GSD type IXa (86).

By use of trio exome sequencing in patients with IKH, researchs have identified variants also in *SLC16A1* (MCT1), *NCOR1*, *IGF2BP1*, *SGLT2* and *NEK11* as potential novel causes of unexplained KH (69, 87, 88).

In the field of GSDs, Kim at al. reported the gene panel for GSDs as a useful tool to confirm the diagnosis of GSD IX subtypes. They clarified genotype, phenotype and long-term outcomes of patients with GSD type IX. Furthermore, they reported the development of hepatocellular carcinoma in a patient with GSD type IXc (42).

Seven new GSD type 0 patients with variable phenotypes were found by a gene panel in a recent report. Seven variants were novel, and four were classified as of uncertain clinical significance (VUS). Their frequency in the healthy cohort was extremely low, but there were not enough supporting criteria for interpreting these variants as pathogenic or likely pathogenic. Predictive systems gave different interpretations. All patients showed hyperglycemia and hyperlactatemia three hours after feeding, with some having ketones in blood and urine, others only in blood. One patient showed enlarged liver (89). In another study, two patients lacking of postprandial hyperglycemia/hyperlactatemia were diagnosed with GSD typo 0 by targeted NGS (1). Another report described a diagnosis of GSD type 0 through WES in a patient with a nonclassic presentation (90). In other two studies exome sequencing was used in pediatric and adult patients with GSDs affecting both liver and/or muscle. The former reported a presumptive diagnostic yield of 65% by targeted exome sequencing (91). In the other, the diagnostic yield was 43% with clinical exome sequencing and 25% with targeted exome sequencing (92).

In the Ponzi study, the use of a gene panel for hypoglycemia showed a mutation detection rate of 59% in GSDs and other carbohydrate metabolism disorder subgroup. A diagnosis of

GSD type IXa was established in a patient with hemizygous deletion in *PHKA2* gene. GDS type III was diagnosed in a patient with homozygous deletion in *AGL* gene. Another child was diagnosed with GSD type IXc, carrying an unreported biallelic missense mutation in *PHKG2* gene which strongly correlated with the observed phenotype. Unexpectedly, two GSD type 0, one HI and one HFI were diagnosed from the no candidate gene class. These three patients presented with unusual findings: variable fasting tolerance, intermittent ketonemia and absence of postprandial hyperglycemia/hyperlactatemia in GSD type 0; hypoketotic hypoglycemia responsive to glucagon, with increased NEFA in HI; absence of fructose containing foods aversion, fasting hypoketotic hypoglycemia responsive to glucagon, suppressed NEFA, increased liver enzymes and renal tubular dysfunction in HFI (1).

Fructose-1,6-phosphatase deficiency is often misdiagnosed. A study described four patients with recurrent hypoglycemia diagnosed *via* targeted NGS panel. In three of them, the onset of hypoglycemia was in the first two years of age. However, without a clear diagnosis, the families were not aware of how to prevent the attacks, thus they experienced several life threatening events until the genetic diagnosis was settled (93). In a recent case report, the trio exome sequencing revealed the diagnosis postmortem ("molecular autopsy") of cytosolic PEPCK deficiency in a 3-year-old boy with an initial suspicion of a febrile seizure during infection, evolved rapidly in hypoglycemia and cerebral edema. The metabolic screening showed elevated urinary lactate and Krebs cycle intermediates, indicating an energy deficiency. The postmortem diagnosis had an important psychosocial impact for the whole family and provided the chance of a predictive testing to all family members at risk (94). Unexplained coma and sudden death in an apparent healthy infant is a dramatic family life event. Several studies reported on high rates of emotional distress in parents of children with undiagnosed conditions (95).

Rojnueangnit et al. described two unrelated infants with atypical presentation which expanded the phenotype of *HMGCS2* deficiency. During acute episodes, steatorrhea and dyslipidemia (increased triglycerides, VLDL, and LDL, along with decreased HDL) occurred, both previously unreported. Both patients manifested encephalopathy, hypophosphatemia, hyperphosphaturia and proteinuria. One patient presented metabolic acidosis without hypoglycemia. The urinary 4-HMP was not detected. Trio WES revealed compound heterozygous for *HMGCS2*. Hypoglycemia with impaired ketogenesis may have determined an increased lipolysis with raise of NEFA along with triglycerides (96). Although the presence of 4-HMP in urine has been reported as a biomarker of *HMGCS2* deficiency (63), the substance is not always present (97, 98) and likely only appears during decompensation (63). Furthermore, the authors showed that metabolic acidosis without hypoglycemia can be a metabolic feature of *HMGCS2* deficiency (96). Elsewhere, the patient with a ketogenesis defect showed homozygosity for a not reported variant in *HMGCS2* captured by gene panel, predicted as pathogenic *in silico*. A revaluation of his urinary organic acid profile confirmed the presence of the characteristic pattern (1).

In the Ponzi study, the 78% of patients with a single candidate gene, 49% of patients with multiple candidate genes, and 33% with no candidate gene reached a diagnosis. The diagnostic yield of the gene panel was 48% for HI, 59% for GSDs and other carbohydrate disorders, 66% per FAODs and ketogenesis defects, and 67% for mitochondrial disorders (1).

5 ROLE OF GENETICS IN FINDINGS NOVEL GENES

WES has been frequently used to map novel genes involved in the pathogenesis of unexplained hypoglycemia. Variants in *SLC16A1* (MCT1), *NCOR1*, *IGF2BP1*, *SGLT2* and *NEK11* have been identified as potential novel causes of unexplained KH (69, 87, 88).

MCT deficiency was described above: inhibiting mutations are responsible for moderate or profound ketosis and sometimes hypoglycemia (69, 70). The other genes are involved in various processes that might affect gluconeogenesis, glycogenolysis and translational regulation.

The NCOR1/HDAC3 complex is involved in the regulation of liver phosphoenolpyruvate carboxykinase (PEPCK), glucose 6-phosphatase catalytic (G6PC) and hepcidin. The KH patient with an *NCOR1* mutation had iron deficiency anemia as additional feature, likely due to hepcidin overexpression (87). Remarkably, patients with GSD type Ia (*G6PC* mutations) may present also iron deficiency anemia caused by increased secretion of hepcidin from hepatic adenomas (30).

IGF2BP1 regulates the activity of several proteins, including IGF2 that is involved in cell growth and differentiation and activates the insulin receptor. *In vitro*, IGF2BP1 suppresses the translation of IGF2 mRNA. Therefore, increased IGF2 levels will be caused by loss of function mutations of *IGFBP2*. Remarkably, impaired glycogenolysis and gluconeogenesis with consequent hypoglycemia and suppressed insulin secretion have been found in patients with IGF2-producing tumors.

Dominant mutations in *SLC5A2*, which encodes the sodium glucose co-transporter 2 (SGLT2), lead to familial renal glucosuria (FRG). The KH patient with a *SLC5A2* mutation presented hypoglycemia with intermittent glucosuria, suggesting that FRG may cause KH in infancy.

The mitosis gene A-related kinase 11 gene *NEK11* has not been linked to other diseases in humans than KH. Hypoglycemia was reported in a *NEK11*- mouse model. The described KH patient with a heterozygous *NEK11* mutation showed glucagon unresponsive hypoglycemia, migraine, cognitive disability, motor impairments, mild hepatopathy, and decreased plasma IGFBP3 (71, 87).

Recently, the use of exome sequencing allowed the detection of a single *CACNA1D* (encoding for the L-type voltage-gated calcium channel) activating mutation in a syndromic child with neurodevelopmental delay, aortic insufficiency and HI requiring diazoxide therapy. As pancreatic L-type voltage-gated calcium channels are involved in insulin secretion, mutations in

CACNA1D may have a potential pathogenic role (99). A previous patient with primary aldosteronism and without HI had been described (100). A second patient with developmental delay, hypotonia, aortic insufficiency, primary hyperaldosteronism, and facial dysmorphisms was recently diagnosed by clinical exome sequencing, identifying a novel *de novo CACNA1D* missense mutation, thus confirming the implication of *CACNA1D* for primary aldosteronism and HI. The genetic diagnosis led to add nifedipine to the therapy that was effective for glycemic and pressure control and muscle tone (99, 101). Nifedipine readily permeates the blood-brain barrier and thus may also inhibit L-type voltage-gated calcium channels in the brain. Indeed, the start of nifedipine was associated with an improvement of hypotonia (101).

A further gene recently likely associated with HI is the developmental transcription factor, forkhead box A2, *FOXA2*, in which a *de novo* heterozygous mutation was found by WES in a child with HI, hypopituitarism, liver, lung and gastrointestinal malformations, choroidal coloboma and dysmorphisms (102). Foxa2 is an important developmental transcription factor for the formation of midline structures and endoderm derived organs including the pancreas, and regulates insulin secretion from pancreatic β-cells. The mechanisms are poorly understood. However, a *FOXA2* mutation might alter the expression of SUR1 and/or Kir6.2, or activate the transcription of *HADH* that encodes L-3-Hydroxyacyl-CoA-dehydrogenase (HADH), an enzyme involved in the penultimate step of the β-oxidation pathway. Furthermore, it could increase the GLUT2 expression in pancreatic β-cells, promoting the glucose transport through the cell membranes and thus enhancing the insulin secretion. Foxa2 could also play a role in the development of the pancreas, through the regulation of *Pdx1*, a homeobox gene essential for pancreatic development. Finally, Foxa2 has also been implicated in the regulation of Hnf4a and Hnf1a, involved in HI and monogenic diabetes (102).

Recent description of loss of function mutations in *EIF2S3*, discovered by exome sequencing of the X-chromosome, were also been associated to hypoglycemia, hypopituitarism and pancreatic dysfunction in three boys. The heterotrimeric GTP-binding protein eIF2 forms a ternary complex with methionyltRNA promoting the onset of protein synthesis. Mutations in *EIF2S3* (X-linked), encoding the eIF2γ subunit, have been associated with cardinal phenotypic features of microcephaly and intellectual disability, usually as part of MEHMO syndrome characterized by short stature, hypogonadism, epilepsy, significant intellectual disability and microcephaly. The three patients presented a novel milder phenotype, with mild learning difficulties, short stature, hypogonadism and glucose dysregulation with HI and post-prandial hyperglycaemia (as shown at prolonged glucose tolerance test with hypoglycemia at baseline and at 5 hours with a detectable insulin, and hyperglycemia at 2 hours). They were treated with rhGH, thyroxine, diazoxide and chlorothiazide. The early molecular diagnosis might have contributed to the prevention of severe neurodevelopmental delay, which could be related to untreated unrecognized hypoglycemia (103).

Recessive homozygous c.4910G>A mutations of *LRP4* have been recently discovered by clinical exome sequencing to be associated to unexplained KH in two siblings affected by Cenani-Lenz syndactyly, characterized by skeletal abnormalities, dysmorphisms, renal hypoplasia, deafness, congenital cataract (104). However, since LRP4 is a receptor involved in cell adhesion and receptor-ligand interactions in bone, kidney and nervous system, its putative role in the pathogenesis of hypoglycemia might be coincidental.

Recessive loss of function mutations of *DNAJC3* have been found by exome and genome sequencing to cause early HI evolving into diabetes for insulin insufficiency, growth retardation and neurodegeneration in four children (105, 106). Patients presented demyelinating neuropathy, learning difficulties, hypothyroidism, microcephaly, retinal dystrophy, sensorineural deafness. DNAJC3 ($P58^{IPK}$) is member of the heat shock proteins produced in the endoplasmic reticulum to counter cell stress and having a protective role against neurodegeneration. The gene is expressed in endocrine cells such as pancreas and thyroid. Early onset HI might be due to a disturbance of the balance between β-cell apoptosis and proliferation. HI is responsive to diazoxide (105). Furthermore, $P58^{IPK}$ null mice developed inhibition of C/ebpα which regulates gluconeogenesis and lipogenesis in the liver. These mice manifested fatty liver, hypoglycemia and depletion of hepatic glycogen (107).

6 DISCUSSION AND CLOSING REMARKS

In the last years the molecular approach for sequencing genetic informations at scale has changed substantially. Historically, genetic analysis consisted on single gene testing at a time. Lately, the new NGS technology made it possible to carry out large molecular characterization of patients within an useful timeframe. This was particularly applicable for the diagnosis of IEM presenting with hypoglycemia, because of the genetic heterogeneity of these conditions (1) (**Figure 1**, **Table 1**).

Targeted gene panels capture variants within a few target genes (10s to 100s of genes) and are commonly used for diagnostic purposes, as they generate manageable quantities of data with a low cost and turnaround time. The list of all genes is pre-established and needs to be updated along with new disease genes discovering (**Table 1**). A further approach is the use of virtual panels to make available a clinical exome (Mendelioma, for sequencing all genes associated to Mendelian inheritance) data set that can be reviewed overtime at no additional cost to test other candidate genes (108, 109). However, in instances of diagnostic uncertainty, WES has a higher diagnostic yield. WES detects all variants within the entire protein-coding region (~20,000 genes), representing less than 2% of the genome but containing the ~ 85% of known disease-causing variants. WES is suitable for novel gene discovery in idiopathic conditions. However, non-coding and structural variants cannot be captured, and the gene coverage may be variable. Therefore, the detection of mutations in deep intronic regions may be missed by the use of gene panels or WES, or if a detected mutation was considered as a nonpathogenic variant. Whole-genome sequencing (WGS) captures variants from the entire genome. Besides revealing the ~98% of the non-coding regions, it provides a better coverage and analysis of the coding regions. Indeed, WGS

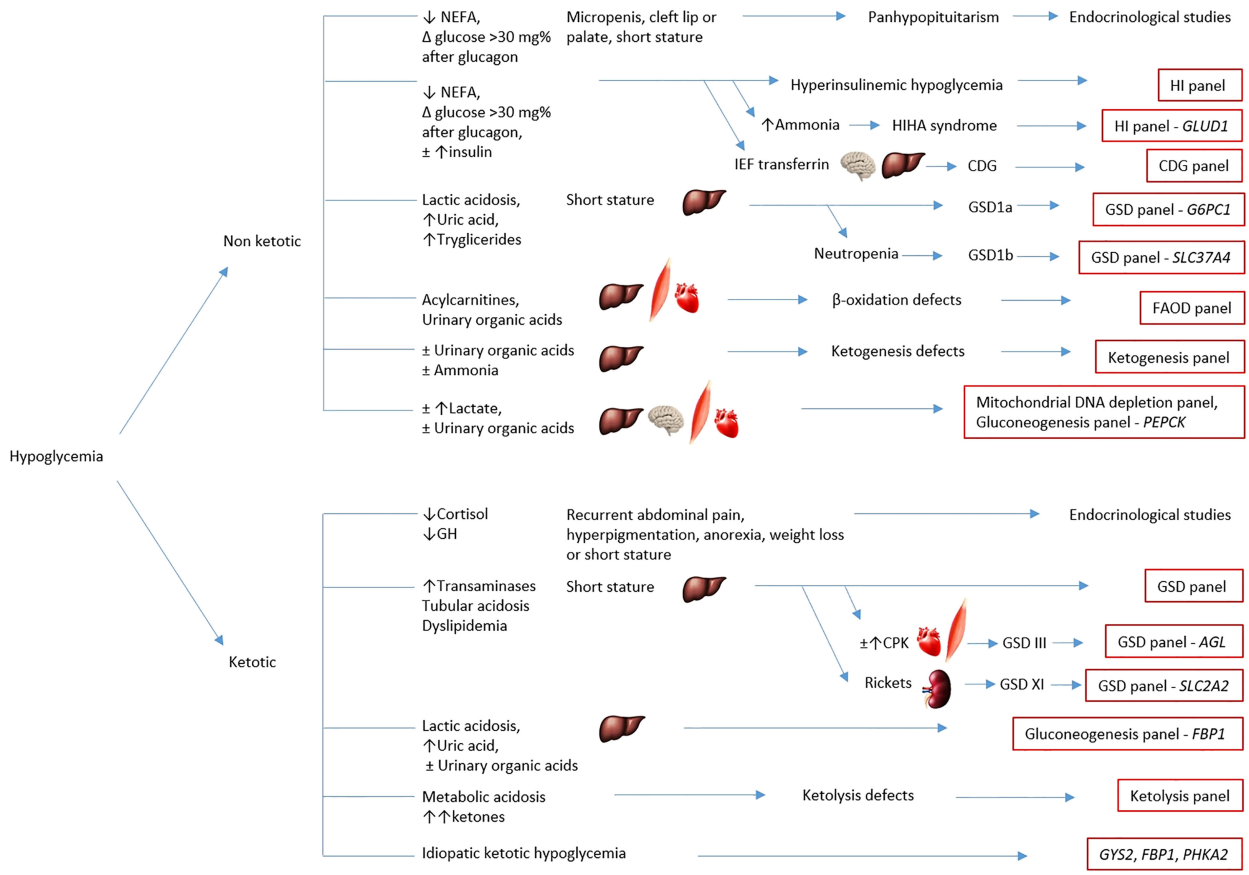

FIGURE 1 | Simplified chart of the diagnostic algorithm for hypoglycemia. The chart summarizes the main causes of hypoglycemia and classifies them according to clinical and biochemical characterization for targeted metabolic gene panel testing. The eventual hepatic, cardiac, muscular or neurological involvement is outlined by the corresponding anatomical pictures. HI, hyperinsulinemic hypoglycemia; HIHA syndrome, hyperinsulinism-hyperammonemia syndrome; CDG, congenital disorders of glycosylation; GSD, glycogen storage disorders; FAOD, fatty acids oxidation defects; IEF transferrin, isoelectrofocusing of transferrin.

revealed large deletions in genes associated to some IEM, not detected by WES. Nevertheless, reliable tools to interpret non-coding variants are still not available, and cost and turnaround time are high (110, 111). However, the buildup of WES and WGS data in the human population and the systematic use of trio sequencing will possibly increase the diagnostic yield in unexplained conditions (79). Though, as WES and WGS lean on short-read sequencing, there are genomic regions still difficult to sequence such as tandem-repeat expansion, large deletions and insertions, and complex chromosomal structural abnormalities. In those cases, long-read sequencing technologies could unveil such rare variations. Recent studies about the application of long-read sequencing to pathogenic variants in rare diseases not detected by conventional NGS techniques gave promising results (112).

A wide proportion of existing variants are classified as VUS, for which functional studies or computational tools are necessary to clarify the pathogenicity. It has been estimated that the probability to detect a VUS is as higher as larger is the number of genes tested: 36% in multigene panels and 73% in exome sequencing (113). To establish a proven genetic diagnosis, a disease causing variant should be detectable and clinically interpretable. Various computational prediction systems have been developed to interpret the impact of those variants on clinical phenotypes (110). However, international guidelines are insufficient to unravel the pathogenicity of certain findings (110, 114). As more information become gradually available, VUS may be redefined as pathogenic/likely pathogenic or benign/likely benign (113). In communicating genetic VUS, there is a risk of overdiagnosis and overtreatment if they were inappropriately classified as pathogenic. Multidisciplinary cooperation is prominent for interpreting the significance of genomic results (111).

In cases of negative or partial NGS results of targeted gene panels and clinical exome sequencing, but strong evidence of biochemical or clinical phenotype or discrepancy with segregation studies, other techniques should be used, including multiple ligation-dependent probe amplification (MLPA) and high-resolution comparative genomic hybridization (CGH) array (1) (**Figure 2**). In a recent case of GSD type III, the use of single nucleotide polymorphism (SNP) array and short tandem repeats (STR) segregation study revealed for the first time a paternal isodisomy of chromosome 1 (115). Conversely, NGS approach can help to make a diagnosis in

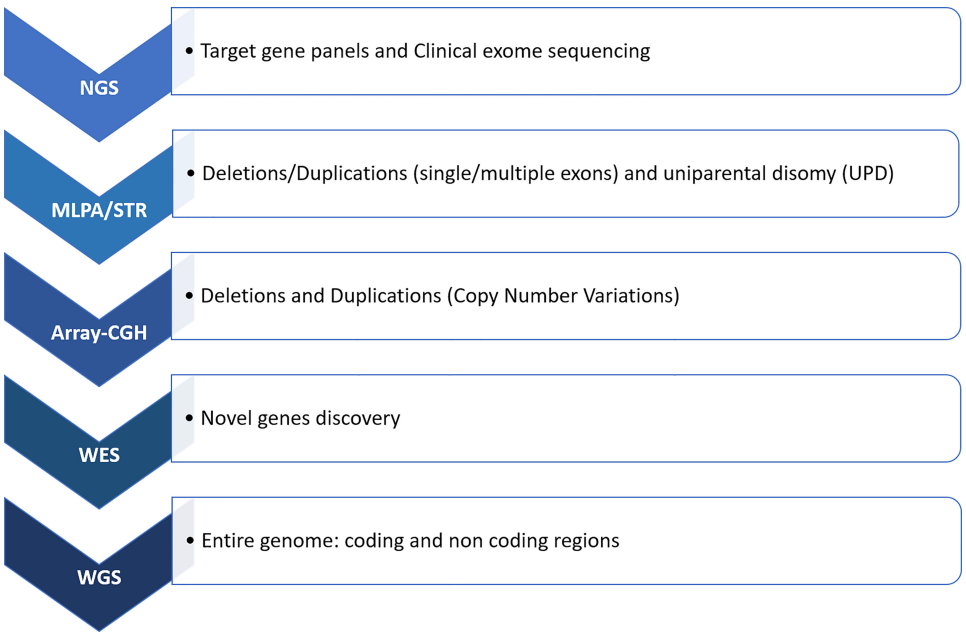

FIGURE 2 | Molecular workflow for hypoglycemic conditions. In cases of not conclusive results from NGS, a research for deletion/duplications should be performed through MLPA and array-CGH. Integration with STR can be worthwhile when an uniparental disomy is suspected. Subsequently, WES and WGS techniques can be used for sequencing the entire genetic code. NGS, next generation sequencing; MLPA, multiple ligation-dependent probe amplification; array-CGH, comparative genomic hybridization array; STR, short tandem repeats; WES, whole exome sequencing; WGS, whole genome sequencing.

case of negative biochemical results as in some GSDs, or in *HMGCS2* mutations in which the characteristic biomarker is not always present (96–98).

The importance to make a rapid differential diagnosis lies in establishing the appropriate therapeutic approach, such as specific nutritional intervention regarding GSDs and FAODs type and GCK-HI, or medical therapy in case of GSD type Ib as well as *HNF4a*/*SLC2A2* gene mutations, which share a similar phenotype but different treatment, or surgical strategies in case of focal-HI.

Identification of new rare disease genes may influence the impact of receiving a diagnosis, because often the long-term effects of new genetic conditions are unknown. The agnostic approach of WES and WGS is also challenging our previous knowledges of existing genetic diagnoses, when pathogenic variants give raise to unexpected clinical pictures. Therefore, this approach is allowing to expand the phenotypic characterization of rare diseases (111), such as the above reported cases of GSD type IXa (85), *HMGCS2* deficiency (96), MEHMO syndrome (103).

By using the NGS approach in IEM presenting with hypoglycemia, Ponzi et al. demonstrated that it provided a rapid diagnosis in 45% of patients in whom clinical and laboratory findings did not allow to identify a single candidate gene. Furthermore, invasive or expensive procedures have been avoided, such as liver biopsy for suspected disorders of carbohydrate metabolism. However, NGS will not replace the metabolic work-up, which is critical to drive the molecular analysis toward those clusters of genes involved in specific pathways (**Figure 1**). In addition, the biochemical and clinical phenotype addresses data interpretation regarding the finding of possible disease-causing variants at first reported as VUS, or the discovery of new disease genes (1). Remarkably, in a huge retrospective study of WES applied as a primary newborn screening test for 48 IEM in an 8.5-year population scale cohort in California (the NBSeq project) an 88% overall sensitivity and 98.4% specificity was estimated. Conversely, the current screening performed with MS/MS analite-based shows 99.0% sensitivity and 99.8% specificity in the same cohort (110). Furthermore, a synergistic association with other biotechnologies, such as enzymatic assay for residual activity in FAODs, may allow a better characterization of new variants in order to define pathogenicity, customize follow-up and avoid overtreatment (116). Finally, the NGS approach allows genetic counseling for recurrence risk in further pregnancies (1), prenatal and preimplantation diagnosis (43).

In conclusion, innovative diagnostic protocols are required for genetically heterogeneous disorders. NGS can routinely be easily used as a standard diagnostic tool with a straightforward workflow to identify disease-causing mutations. It allows an early detection of mutations, with a high standard in terms of coverage and quality, is cost-effective and has a rapid turnaround time. These data promote the expanding application of the NGS technologies for IEM presenting with hypoglycemia, because of their genetic heterogeneity and complex phenotypes, variable or atypical presentations, for a more appropriate clinical management and genetic counselling (79). Multidisciplinary input and collaboration are increasingly key for addressing the analysis and interpreting the significance of the genetic results, allowing rapidly their translation from bench to bedside.

AUTHOR CONTRIBUTIONS

AM conceptualized and designed the study, searched for literature, drafted the initial manuscript, reviewed and approved the final manuscript as submitted. FL, AN, and CD-V wrote sections of the manuscript, critically reviewed the manuscript and approved the final manuscript as submitted. All authors contributed to manuscript revision, read, and approved the submitted version.

REFERENCES

1. Ponzi, E, Maiorana, A, Lepri, FR, Mucciolo, M, Semeraro, M, Taurisano, R, et al. Persistent Hypoglycemia in Children: Targeted Gene Panel Improves the Diagnosis of Hypoglycemia Due to Inborn Errors of Metabolism. *J Pediatr* (2018) 202:272–8.e4. doi: 10.1016/j.jpeds.2018.06.050
2. Ghosh, A, Banerjee, I, Morris, AA. Recognition, Assessment and Management of Hypoglycaemia in Childhood. *Arch Dis Child* (2016) 101:575–80. doi: 10.1136/archdischild-2015-308337
3. Thornton, PS, Stanley, CA, De Leon, DD, Harris, D, Haymond, MW, Hussain, K, et al. Recommendations From the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. *J Pediatr* (2015) 167:238–45. doi: 10.1016/j.jpeds.2015.03.057
4. Arnoux, JB, Verkarre, V, Saint-Martin, C, Montravers, F, Brassier, A, Valayannopoulos, V, et al. Congenital Hyperinsulinism: Current Trends in Diagnosis and Therapy. *Orphanet J Rare Dis* (2011) 6:63. doi: 10.1186/1750-1172-6-63
5. De Leon, DD, Stanley, CA. Determination of Insulin for the Diagnosis of Hyperinsulinemic Hypoglycemia. *Best Pract Res Clin Endocrinol Metab* (2013) 27:763–9. doi: 10.1016/j.beem.2013.06.005
6. Ferrara, C, Patel, P, Becker, S, Stanley, CA, Kelly, A. Biomarkers of Insulin for the Diagnosis of Hyperinsulinemic Hypoglycemia in Infants and Children. *J Pediatr* (2016) 168:212–9. doi: 10.1016/j.jpeds.2015.09.045
7. De Leon, DD, Stanley, CA. Congenital Hypoglycemia Disorders: New Aspects of Etiology, Diagnosis, Treatment and Outcomes: Highlights of the Proceedings of the Congenital Hypoglycemia Disorders Symposium, Philadelphia April 2016. *Pediatr Diabetes* (2017) 18:3–9. doi: 10.1111/pedi.12453
8. Kapoor, RR, Flanagan, SE, Arya, VB, Shield, JP, Ellard, S, Hussain, K. Clinical and Molecular Characterisation of 300 Patients With Congenital Hyperinsulinism. *Eur J Endocrinol* (2013) 168:557–64. doi: 10.1530/EJE-12-0673
9. Faletra, F, Athanasakis, E, Morgan, A, Biarnés, X, Fornasier, F, Parini, R, et al. Congenital Hyperinsulinism: Clinical and Molecular Analysis of a Large Italian Cohort. *Gene* (2013) 521:160–5. doi: 10.1016/j.gene.2013.03.021
10. Flanagan, SE, Clauin, S, Bellanné-Chantelot, C, de Lonlay, P, Harries, LW, Gloyn, AL, et al. Update of Mutations in the Genes Encoding the Pancreatic Beta-Cell K(ATP) Channel Subunits Kir6.2 (KCNJ11) and Sulfonylurea Receptor 1 (ABCC8) in Diabetes Mellitus and Hyperinsulinism. *Hum Mutat* (2009) 30:170–80. doi: 10.1002/humu.20838
11. Snider, KE, Becker, S, Boyajian, L, Shyng, S-L, MacMullen, C, Hughes, C, et al. Genotype and Phenotype Correlations in 417 Children With Congenital Hyperinsulinism. *J Clin Endocrinol Metab* (2013) 98:E355–363. doi: 10.1210/jc.2012-2169
12. De Lonlay, P, Fournet, JC, Rahier, J, Gross-Morand, MS, Poggi-Travert, F, Foussier, V, et al. Somatic Deletion of the Imprinted 11p15 Region in Sporadic Persistent Hyperinsulinemic Hypoglycemia of Infancy is Specific of Focal Adenomatous Hyperplasia and Endorses Partial Pancreatectomy. *J Clin Invest* (1997) 100:802–7. doi: 10.1172/JCI119594
13. Maiorana, A, Dionisi-Vici, C. Hyperinsulinemic Hypoglycemia: Clinical, Molecular and Therapeutical Novelties. *J Inherit Metab Dis* (2017) 40(4):531–42. doi: 10.1007/s10545-017-0059-x
14. Stanley, CA. Perspective on the Genetics and Diagnosis of Congenital Hyperinsulinism Disorders. *J Clin Endocrinol Metab* (2016) 101:815–26. doi: 10.1210/jc.2015-3651
15. de Lonlay-Debeney, P, Poggi-Travert, F, Fournet, JC, Sempoux, C, Dionisi Vici, C, Brunelle, F, et al. Clinical Features of 52 Neonates With Hyperinsulinism. *N Engl J Med* (1999) 340:1169–75. doi: 10.1056/NEJM199904153401505
16. Beltrand, J, Caquard, M, Arnoux, JB, Laborde, K, Velho, G, Verkarre, V, et al. Glucose Metabolism in 105 Children and Adolescents After Pancreatectomy for Congenital Hyperinsulinism. *Diabetes Care* (2012) 35:198–203. doi: 10.2337/dc11-1296
17. Arya, VB, Senniappan, S, Demirbilek, H, Alam, S, Flanagan, SE, Ellard, S, et al. Pancreatic Endocrine and Exocrine Function in Children Following Near-Total Pancreatectomy for Diffuse Congenital Hyperinsulinism. *PloS One* (2014) 9:e98054. doi: 10.1371/journal.pone.0098054
18. Lord, K, Radcliffe, J, Gallagher, PR, Adzick, NS, Stanley, CA, De Leon, DD. High Risk of Diabetes and Neurobehavioral Deficits in Individuals With Surgically Treated Hyperinsulinism. *J Clin Endocrinol Metab* (2015) 100:4133–9. doi: 10.1210/jc.2015-2539
19. Maiorana, A, Caviglia, S, Greco, B, Alfieri, P, Cumbo, F, Campana, C, et al. Ketogenic Diet as Elective Treatment in Patients With Drug-Unresponsive Hyperinsulinemic Hypoglycemia Caused by Glucokinase Mutations. *Orphanet J Rare Dis* (2021) 16:424. doi: 10.1186/s13023-021-02045-3
20. Stanescu, DE, Hughes, N, Kaplan, B, Stanley, CA, De Leon, DD. Novel Presentations of Congenital Hyperinsulinism Due to Mutations in the MODY Genes: HNF1A and HNF4A. *J Clin Endocrinol Metab* (2012) 97:E2026–30. doi: 10.1210/jc.2012-1356
21. Stanik, J, Skopkova, M, Brennerova, K, Danis, D, Rosolankova, M, Salingova, A, et al. Congenital Hyperinsulinism and Glycogenosis-Like Phenotype Due to a Novel HNF4A Mutation. *Diabetes Res Clin Pract* (2017) 126:144–50. doi: 10.1016/j.diabres.2017.02.014
22. Shanti, B, Silink, M, Bhattacharya, K, Howard, NJ, Carpenter, K, Fietz, M, et al. Congenital Disorder of Glycosylation Type Ia: Heterogeneity in the Clinical Presentation From Multivisceral Failure to Hyperinsulinaemic Hypoglycaemia as Leading Symptoms in Three Infants With Phosphomannomutase Deficiency. *J Inherit Metab Dis* (2009) 32(Suppl 1):S241–51. doi: 10.1007/s10545-009-1180-2
23. Čechová, A, Altassan, R, Borgel, D, Bruneel, A, Correia, J, Girard, M, et al. Consensus Guideline for the Diagnosis and Management of Mannose Phosphate Isomerase-Congenital Disorder of Glycosylation. *J Inherit Metab Dis* (2020) 43:671–93. doi: 10.1002/jimd.12241
24. Miller, BS, Freeze, HH, Hoffmann, GF, Sarafoglou, K. Pubertal Development in ALG6 Deficiency (Congenital Disorder of Glycosylation Type Ic). *Mol Genet Metab* (2011) 103:101–3. doi: 10.1016/j.ymgme.2011.01.016
25. Sun, L, Eklund, EA, Chung, WK, Wang, C, Cohen, J, Freeze, HH. Congenital Disorder of Glycosylation Id Presenting With Hyperinsulinemic Hypoglycemia and Islet Cell Hyperplasia. *J Clin Endocrinol Metab* (2005) 907:4371–5. doi: 10.1210/jc.2005-0250
26. Tegtmeyer, LC, Rust, S, van Scherpenzeel, M, Ng, BG, Losfeld, ME, Timal, S, et al. Multiple Phenotypes in Phosphoglucomutase 1 Deficiency. *N Engl J Med* (2014) 370:533–42. doi: 10.1056/NEJMoa1206605
27. Altassan, R, Radenkovic, S, Edmondson, AC, Barone, R, Brasil, S, Cechova, A, et al. International Consensus Guidelines for Phosphoglucomutase 1 Deficiency (PGM1-CDG): Diagnosis, Follow-Up, and Management. *J Inherit Metab Dis* (2021) 44:148–63. doi: 10.1002/jimd.12286
28. Kostopoulou, E, Dastamani, A, Güemes, M, Clement, E, Caiulo, S, Shanmugananda, P, et al. Syndromic Forms of Hyperinsulinaemic Hypoglycaemia-A 15-Year Follow-Up Study. *Clin Endocrinol (Oxf)* (2021) 94:399–412. doi: 10.1111/cen.14393
29. Derks, TGJ, Rodriguez-Buritica, DF, Ahmad, A, de Boer, F, Couce, ML, Grünert, SC, et al. Glycogen Storage Disease Type Ia: Current Management Options, Burden and Unmet Needs. *Nutrients* (2021) 13:3828. doi: 10.3390/nu13113828
30. Kishnani, PS, Austin, SL, Abdenur, JE, Arn, P, Bali, DS, Boney, A, et al. Diagnosis and Management of Glycogen Storage Disease Type I: A Practice Guideline of the American College of Medical Genetics and Genomics. *Genet Med* (2014) 16:e1. doi: 10.1038/gim.2014.128

31. Wortmann, SB, Van Hove, JLK, Derks, TGJ, Chevalier, N, Knight, V, Koller, A, et al. Treating Neutropenia and Neutrophil Dysfunction in Glycogen Storage Disease Type Ib With an SGLT2 Inhibitor. *Blood* (2020) 136:1033–43. doi: 10.1182/blood.2019004465
32. Kishnani, PS, Austin, SL, Arn, P, Bali, DS, Boney, A, Case, LE, et al. Glycogen Storage Disease Type III Diagnosis and Management Guidelines. *Genet Med* (2010) 12:446–63. doi: 10.1097/GIM.0b013e3181e655b6
33. Valayannopoulos, V, Bajolle, F, Arnoux, JB, Dubois, S, Sannier, N, Baussan, C, et al. Successful Treatment of Severe Cardiomyopathy in Glycogen Storage Disease Type III With D, L-3-Hydroxybutyrate, Ketogenic and High Protein Diet. *Pediatr Res* (2011) 70:638–41. doi: 10.1203/PDR.0b013e318232154f
34. Mayorandan, S, Meyer, U, Hartmann, H, Das, AM. Glycogen Storage Disease Type III: Modified Atkins Diet Improves Myopathy. *Orphanet J Rare Dis* (2014) 9:196. doi: 10.1186/s13023-014-0196-3
35. Brambilla, A, Mannarino, S, Pretese, R, Gasperini, S, Galimberti, C, Parini, R. Improvement of Cardiomyopathy After High-Fat Diet in Two Siblings With Glycogen Storage Disease Type III. *JIMD Rep* (2014) 17:91–5. doi: 10.1007/8904_2014_343
36. Rossi, A, Hoogeveen, IJ, Bastek, VB, de Boer, F, Montanari, C, Meyer, U, et al. Dietary Lipids in Glycogen Storage Disease Type III: A Systematic Literature Study, Case Studies, and Future Recommendations. *J Inherit Metab Dis* (2020) 43:770–7. doi: 10.1002/jimd.12224
37. Derks, TGJ, Peeks, F, de Boer, F, Fokkert-Wilts, M, van der Doef, HPJ, van den Heuvel, MC, et al. The Potential of Dietary Treatment in Patients With Glycogen Storage Disease Type IV. *J Inherit Metab Dis* (2021) 44:693–704. doi: 10.1002/jimd.12339
38. Aeppli, TR, Rymen, D, Allegri, G, Bode, PK, Häberle, J. Glycogen Storage Disease Type VI: Clinical Course and Molecular Background. *Eur J Pediatr* (2020) 179:405–13. doi: 10.1007/s00431-019-03499-1
39. Burwinkel, B, Bakker, HD, Herschkovitz, E, Moses, SW, Shin, YS, Kilimann, MW, et al. Mutations in the Liver Glycogen Phosphorylase Gene (Pygl) Underlying Glycogenosis(Hers Disease). *Am J Hum Genet* (1998) 62:785–91. doi: 10.1086/301790
40. Chang, S, Rosenberg, MJ, Morton, H, Francomano, CA, Biesecker, LG. Identification of a Mutation in Liver Glycogen Phosphorylase in Glycogen Storage Disease Type VI. *Hum Mol Genet* (1998) 7:865–70. doi: 10.1093/hmg/7.5.865
41. Luo, X, Hu, J, Gao, X, Fan, Y, Sun, Y, Gu, X, et al. Novel PYGL Mutations in Chinese Children Leading to Glycogen Storage Disease Type VI: Two Case Reports. *BMC Med Genet* (2020) 21:74. doi: 10.1186/s12881-020-01010-4
42. Kim, TH, Kim, KY, Kim, MJ, Seong, MW, Park, SS, Moon, JS, et al. Molecular Diagnosis of Glycogen Storage Disease Type IX Using a Glycogen Storage Disease Gene Panel. *Eur J Med Genet* (2020) 63:103921. doi: 10.1016/j.ejmg.2020.103921
43. Eghbali, M, Fatemi, KS, Salehpour, S, Abiri, M, Saei, H, Talebi, S, et al. Whole-Exome Sequencing Uncovers Novel Causative Variants and Additional Findings in Three Patients Affected by Glycogen Storage Disease Type VI and Fanconi–Bickel Syndrome. *Front Genet* (2020) 11:601566. doi: 10.3389/fgene.2020.601566
44. Sharari, S, Abou-Alloul, M, Hussain, K, Ahmad Khan, F. Fanconi–Bickel Syndrome: A Review of the Mechanisms That Lead to Dysglycaemia. *Int J Mol Sci* (2020) 21:6286. doi: 10.3390/ijms21176286
45. Weinstein, DA, Correia, CE, Saunders, AC, Wolfsdorf, JI. Hepatic Glycogen Synthase Deficiency: An Infrequently Recognized Cause of Ketotic Hypoglycemia. *Mol Genet Metab* (2006) 87:284–8. doi: 10.1016/j.ymgme.2005.10.006
46. Bhattacharya, K. Investigation and Management of the Hepatic Glycogen Storage Diseases. *Transl Pediatr* (2015) 4:240–8. doi: 10.3978/j.issn.2224-4336.2015.04.07
47. Kasapkara, ÇS, Aycan, Z, Açoglu, E, Senel, S, Oguz, MM, Ceylaner, S. The Variable Clinical Phenotype of Three Patients With Hepatic Glycogen Synthase Deficiency. *J Pediatr Endocrinol Metab* (2017) 30:459–62. doi: 10.1515/jpem-2016-0317
48. Heller, S, Worona, L, Consuelo, A. Nutritional Therapy for Glycogen Storage Diseases. *J Pediatr Gastroenterol Nutr* (2008) 47:15–21. doi: 10.1097/MPG.0b013e3181818ea5
49. Petersen, MC, Vatner, DF, Shulman, GI. Regulation of Hepatic Glucose Metabolism in Health and Disease. *Nat Rev Endocrinol* (2017) 13:572e587. doi: 10.1038/nrendo.2017.80
50. Krywawych, S, Katz, G, Lawson, AM, Wyatt, S, Brenton, DP. Glycerol-3-Phosphate Excretion in Fructose-1,6-Diphosphatase Deficiency. *J Inherit Metab Dis* (1986) 9:388–92. doi: 10.1007/BF01800490
51. Baker, L, Winegrad, AI. Fasting Hypoglycaemia and Metabolic Acidosis Associated With Deficiency of Hepatic Fructose-1,6-Diphosphatase Activity. *Lancet* (1970) II:13–6. doi: 10.1016/s0140-6736(70)92474-8
52. Sjarif, DR, Sinke, RJ, Duran, M, Beemer, FA, Kleijer, WJ, Ploos van Amstel, JK, et al. Clinical Heterogeneity and Novel Mutations in the Gene in Three Families With Isolated Glycerol Kinase Deficiency. *J Med Genet* (1998) 35:650–6. doi: 10.1136/jmg.35.8.650
53. Vidnes, J, Sovik, O. Gluconeogenesis in Infancy and Childhood. III. Deficiency of the Extramitochondrial Form of Hepatic Phosphoenolpyruvate Carboxykinase in a Case of Persistent Neonatal Hypoglycaemia. *Acta Paediatr Scand* (1976) 65:307–12. doi: 10.1111/j.1651-2227.1976.tb04890.x
54. Leonard, JV, Hyland, K, Furukawa, N, Clayton, PT. Mitochondrial Phosphoenolpyruvate Carboxykinase Deficiency. *Eur J Pediatr* (1991) 150:198–9. doi: 10.1007/BF01963566
55. Vieira, P, Nagy, II, Rahikkala, E, Väisänen, ML, Latva, K, Kaunisto, K, et al. Cytosolic Phosphoenolpyruvate Carboxykinase Deficiency: Expanding the Clinical Phenotype and Novel Laboratory Findings. *J Inherit Metab Dis Oct* (2021) 8:1–12. doi: 10.1002/jimd.12446
56. Cox, TM. Aldolase B and Fructose Intolerance. *FASEB J* (1994) 8:62–71. doi: 10.1096/fasebj.8.1.8299892
57. Mock, DM, Perman, JA, Thaler, M, Morris, RC Jr. Chronic Fructose Intoxication After Infancy in Children With Hereditary Fructose Intolerance. A Cause of Growth Retardation. *N Engl J Med* (1983) 309:764–70. doi: 10.1056/NEJM198309293091305
58. Maiorana, A, Sabia, A, Corsetti, T, Dionisi-Vici, C. Safety of Vaccines Administration in Hereditary Fructose Intolerance. *Orphanet J Rare Dis* (2020) 15:274. doi: 10.1186/s13023-020-01552-z
59. Demirbas, D, Coelho, AI, Rubio-Gozalbo, ME, Berry, GT. Hereditary Galactosemia. *Metabolism* (2918) 83:188–96. doi: 10.1016/j.metabol.2018.01.025
60. Ruiz-Sala, P, Peña-Quintana, L. Biochemical Markers for the Diagnosis of Mitochondrial Fatty Acid Oxidation Diseases. *J Clin Med* (2021) 10 (21):4855. doi: 10.3390/jcm10214855
61. Morris, AA, Lascelles, CV, Olpin, SE, Lake, BD, Leonard, JV, Quant, PA. Hepatic Mitochondrial 3-Hydroxy-3-Methylglutaryl-Coenzyme a Synthase Deficiency. *Pediatr Res* (1998) 44(3):392–6. doi: 10.1203/00006450-199809000-00021
62. Ramos, M, Menao, S, Arnedo, M, Puisac, B, Gil-Rodríguez, MC, Teresa-Rodrigo, ME, et al. New Case of Mitochondrial HMG-CoA Synthase Deficiency. Functional Analysis of Eight Mutations. *Eur J Med Genet* (2013) 56(8):411–5. doi: 10.1016/j.ejmg.2013.05.008
63. Pitt, JJ, Peters, H, Boneh, A, Yaplito-Lee, J, Wieser, S, Hinderhofer, K, et al. Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency: Urinary Organic Acid Profiles and Expanded Spectrum of Mutations. *J Inherit Metab Dis* (2015) 38(3):459–66. doi: 10.1007/s10545-014-9801-9
64. Gibson, KM, Breuer, J, Nyhan, WL. 3-Hydroxy-3-Methylglutaryl-Coenzyme A Lyase Deficiency: Review of 18 Reported Patients. *Eur J Pediatr* (1988) 148:180–6. doi: 10.1007/BF00441397
65. Grünert, SC, Schlatter, SM, Schmitt, RN, Gemperle-Britschgi, C, Mrázová, L, Balci, MC. 3-Hydroxy-3-Methylglutaryl-Coenzyme A Lyase Deficiency: Clinical Presentation and Outcome in a Series of 37 Patients. *Mol Genet Metab* (2017) 121:206–15. doi: 10.1016/j.ymgme.2017.05.014
66. Duran, M, Ketting, D, Wadman, SK, Jakobs, C, Schutgens, RB, Veder, HA. Organic Acid Excretion in a Patient With 3-Hydroxy-3-Methylglutaryl-Coa Lyase Deficiency: Facts and Artefacts. *Clin Chim Acta Int J Clin Chem* (1978) 90:187–93. doi: 10.1016/0009-8981(78)90521-1
67. Fukao, T, Sasai, H, Aoyama, Y, Otsuka, H, Ago, Y, Matsumoto, H, et al. Recent Advances in Understanding Beta-Ketothiolase (Mitochondrial Acetoacetyl-CoA Thiolase, T2) Deficiency. *J Hum Genet* (2019) 64:99–111. doi: 10.1038/s10038-018-0524-x
68. Lin, Y, Yang, Z, Yang, C, Hu, H, He, H, Niu, T, et al. C4OH is a Potential Newborn Screening Marker-a Multicenter Retrospective Study of Patients With Beta-Ketothiolase Deficiency in China. *Orphanet J Rare Dis* (2021) 16:224. doi: 10.1186/s13023-021-01859-5

69. van Hasselt, PM, Ferdinandusse, S, Monroe, GR, Ruiter, JP, Turkenburg, M, Geerlings, MJ, et al. Monocarboxylate Transporter 1 Deficiency and Ketone Utilization. *N Engl J Med* (2014) 371(20):1900–7. doi: 10.1056/NEJMoa1407778
70. Al-Khawaga, S, AlRayahi, J, Khan, F, Saraswathi, S, Hasnah, R, Haris, B, et al. A SLC16A1 Mutation in an Infant With Ketoacidosis and Neuroimaging Assessment: Expanding the Clinical Spectrum of MCT1 Deficiency. *Front Pediatr* (2019) 7:299. doi: 10.3389/fped.2019.00299
71. Drachmann, D, Hoffmann, E, Carrigg, A, Davis-Yates, B, Weaver, V, Thornton, P, et al. Towards Enhanced Understanding of Idiopathic Ketotic Hypoglycemia: A Literature Review and Introduction of the Patient Organization, Ketotic Hypoglycemia International. *Orphanet J Rare Dis* (2021) 16:173. doi: 10.1186/s13023-021-01797-2
72. Baumgartner, MR, Hörster, F, Dionisi-Vici, C, Haliloglu, G, Karall, D, Chapman, KA, et al. Proposed Guidelines for the Diagnosis and Management of Methylmalonic and Propionic Acidemia. *Orphanet J Rare Dis* (2014) 9:130. doi: 10.1186/s13023-014-0130-8
73. D'Annibale, OM, Koppes, EA, Alodaib, AN, Kochersperger, C, Karunanidhi, A, Mohsen, AW, et al. Characterization of Variants of Uncertain Significance in Isovaleryl-CoA Dehydrogenase Identified Through Newborn Screening: An Approach for Faster Analysis. *Mol Genet Metab* (2021) 134:29–36. doi: 10.1016/j.ymgme.2021.08.012
74. Strauss, KA, Carson, VJ, Soltys, K, Young, ME, Bowser, LE, Puffenberger, EG, et al. Branched-Chain α-Ketoacid Dehydrogenase Deficiency (Maple Syrup Urine Disease): Treatment, Biomarkers, and Outcomes. *Mol Genet Metab* (2020) 129:193–206. doi: 10.1016/j.ymgme.2020.01.006
75. Mayorandan, S, Meyer, U, Gokcay, G, Segarra, NG, de Baulny, HO, van Spronsen, F, et al. Cross-Sectional Study of 168 Patients With Hepatorenal Tyrosinaemia and Implications for Clinical Practice. *Orphanet J Rare Dis* (2014) 9:107. doi: 10.1186/s13023-014-0107-7
76. Staufner, C, Lindner, M, Dionisi-Vici, C, Freisinger, P, Dobbelaere, D, Douillard, C, et al. Adenosine Kinase Deficiency: Expanding the Clinical Spectrum and Evaluating Therapeutic Options. *J Inherit Metab Dis* (2016) 39:273–83. doi: 10.1007/s10545-015-9904-y
77. Shimura, M, Kuranobu, N, Ogawa-Tominaga, M, Akiyama, N, Sugiyama, Y, Ebihara, T, et al. Clinical and Molecular Basis of Hepatocerebral Mitochondrial DNA Depletion Syndrome in Japan: Evaluation of Outcomes After Liver Transplantation. *Orphanet J Rare Dis* (2020) 15:169. doi: 10.1186/s13023-020-01441-5
78. Naviaux, RK, Nguyen, KV. POLG Mutations Associated With Alpers Syndrome and Mitochondrial DNA Depletion. *Ann Neurol* (2005) 58:491. doi: 10.1002/ana.20544
79. Viscomi, C, Zeviani, M. MtDNA-Maintenance Defects: Syndromes and Genes. *J Inherit Metab Dis* (2017) 40:587–99. doi: 10.1007/s10545-017-0027-5
80. Doğulu, N, Tuna Kırsaçlıoğlu, C, Köse, E, Ünlüsoy Aksu, A, Kuloğlu, Z, Kansu, A, et al. The Clinical Variations and Diagnostic Challenges of Deoxyguanosine Kinase Deficiency: A Descriptive Case Series. *J Pediatr Endocrinol Metab* (2021) 34:1341–7. doi: 10.1515/jpem-2021-0108
81. Carrozzo, R, Verrigni, D, Rasmussen, M, de Coo, R, Amartino, H, Bianchi, M, et al. Succinate-CoA Ligase Deficiency Due to Mutations in SUCLA2 and SUCLG1: Phenotype and Genotype Correlations in 71 Patients. *J Inherit Metab Dis* (2016) 39:243–52. doi: 10.1007/s10545-015-9894-9
82. Yohe, S, Hauge, A, Bunjer, K, Kemmer, T, Bower, M, Schomaker, M, et al. Clinical Validation of Targeted Next-Generation Sequencing for Inherited Disorders. *Arch Pathol Lab Med* (2015) 139:204–10. doi: 10.5858/arpa.2013-0625-OA
83. Senior, B. Ketotic Hypoglycemia. A Tale (Tail) of Gauss? *J Pediatr* (1973) 82:555–6. doi: 10.1016/s0022-3476(73)80152-0
84. Morris, AA, Leonard, JV. Early Recognition of Metabolic Decompensation. *Arch Dis Child* (1997) 76(6):555–6. doi: 10.1136/adc.76.6.555
85. Brown, LM, Corrado, MM, van der Ende, RM, Derks, TG, Chen, MA, Siegel, S, et al. Evaluation of Glycogen Storage Disease as a Cause of Ketotic Hypoglycemia in Children. *J Inherit Metab Dis* (2015) 38:489–93. doi: 10.1007/s10545-014-9744-1
86. Ago, Y, Sugie, H, Fukuda, T, Otsuka, H, Sasai, H, Nakama, M, et al. A Rare PHKA2 Variant (P.G991A) Identified in a Patient With Ketotic Hypoglycemia. *JIMD Rep* (2019) 48:15–8. doi: 10.1002/jmd2.12041
87. Alhaidan, Y, Larsen, MJ, Schou, AJ, Stenlid, MH, Al Balwi, MA, Christesen, HT, et al. Exome Sequencing Revealed DNA Variants in NCOR1, IGF2BP1, SGLT2 and NEK11 as Potential Novel Causes of Ketotic Hypoglycemia in Children. *Sci Rep* (2020) 10:2114. doi: 10.1038/s41598-020-58845-3
88. Kaplowitz, P, Sekizkardes, H. Clinical and Laboratory Characteristics and Follow Up of 62 Cases of Ketotic Hypoglycemia: A Retrospective Study. *Int J Pediatr Endocrinol* (2019) 2019:3. doi: 10.1186/s13633-019-0066-9
89. Kamenets, EA, Gusarova, EA, Milovanova, NV, Itkis, YS, Strokova, TV, Melikyan, MA, et al. Hepatic Glycogen Synthase (GYS2) Deficiency: Seven Novel Patients and Seven Novel Variants. *JIMD Rep* (2020) 53(1):39–44. doi: 10.1002/jmd2.12082
90. Szymańska, E, Rokicki, D, Wątrobinska, U, Ciara, E, Halat, P, Płoski, R, et al. Pediatric Patient With Hyperketotic Hypoglycemia Diagnosed With Glycogen Synthase Deficiency Due to the Novel Homozygous Mutation in GYS2. *Mol Genet Metab Rep* (2015) 4:83–6. doi: 10.1016/j.ymgmr.2015.07.003
91. Wang, J, Cui, H, Lee, NC, Hwu, WL, Chien, YH, Craigen, WJ, et al. Clinical Application of Massively Parallel Sequencing in the Molecular Diagnosis of Glycogen Storage Diseases of Genetically Heterogeneous Origin. *Genet Med* (2013) 15:106–14. doi: 10.1038/gim.2012.104
92. Vega, AI, Medrano, C, Navarrete, R, Desviat, LR, Merinero, B, Rodríguez-Pombo, P, et al. Molecular Diagnosis of Glycogen Storage Disease and Disorders With Overlapping Clinical Symptoms by Massive Parallel Sequencing. *Genet Med* (2016) 18:1037–43. doi: 10.1038/gim.2015.217
93. Li, N, Chang, G, Xu, Y, Ding, Y, Li, G, Yu, T, et al. Clinical and Molecular Characterization of Patients With Fructose 1,6-Bisphosphatase Deficiency. *Int J Mol Sci* (2017) 18:857. doi: 10.3390/ijms18040857
94. Becker, J, Haas, NA, Vlaho, S, Heineking, B, Wortmann, SB, Rabenhorst, D, et al. Cytosolic Phosphoenolpyruvate Carboxykinase Deficiency: Cause of Hypoglycemia-Induced Seizure and Death. *Neuropediatrics* (2021) 52:398–402. doi: 10.1055/s-0040-1722685
95. Carmichael, N, Tsipis, J, Windmueller, G, Mandel, L, Estrella, E. "Is it Going to Hurt?": The Impact of the Diagnostic Odyssey on Children and Their Families. *J Genet Couns* (2015) 24:325–35. doi: 10.1007/s10897-014-9773-9
96. Rojnueangnit, K, Maneechai, P, Thaweekul, P, Piriyanon, P, Khositseth, S, Ittiwut, C, et al. Expanding Phenotypic and Mutational Spectra of Mitochondrial HMG-CoA Synthase Deficiency. *Eur J Med Genet* (2020) 63:104086. doi: 10.1016/j.ejmg.2020.104086
97. Conboy, E, Vairo, F, Schultz, M, Agre, K, Ridsdale, R, Deyle, D, et al. Mitochondrial 3-Hydroxy-3-Methylglutaryl-CoA Synthase Deficiency: Unique Presenting Laboratory Values and a Review of Biochemical and Clinical Features. *JIMD Rep* (2018) 40:63–9. doi: 10.1007/8904_2017_59
98. Puisac, B, Marcos-Alcalde, I, Hernandez-Marcos, M, Tobajas Morlana, P, Levtova, A, Schwahn, BC, et al. Human Mitochondrial HMG-CoA Synthase Deficiency: Role of Enzyme Dimerization Surface and Characterization of Three New Patients. *Int J Mol Sci* (2018) 19:1010. doi: 10.3390/ijms19041010
99. Flanagan, SE, Vairo, F, Johnson, MB, Caswell, R, Laver, TW, Lango Allen, H, et al. CACNA1D Mutation in a Patient With Persistent Hyperinsulinaemic Hypoglycaemia, Heart Defects, and Severe Hypotonia. *Pediatr Diabetes* (2017) 18:320–3. doi: 10.1111/pedi.12512
100. Scholl, UI, Goh, G, Stolting, G, de Oliveira, RC, Choi, M, Overton, JD, et al. Somatic and Germline CACNA1D Calcium Channel Mutations in Aldosterone-Producing Adenomas and Primary Aldosteronism. *Nat Genet* (2013) 45:1050–4. doi: 10.1038/ng.2695
101. De Mingo Alemany, MC, Mifsud Grau, L, Moreno Macián, F, Ferrer Lorente, B, León Cariñena, S. A *De Novo* CACNA1D Missense Mutation in a Patient With Congenital Hyperinsulinism, Primary Hyperaldosteronism and Hypotonia. *Channels (Austin)* (2020) 14:175–80. doi: 10.1080/19336950.2020.1761171
102. Giri, D, Vignola, ML, Gualtieri, A, Scagliotti, V, McNamara, P, Peak, M, et al. Novel FOXA2 Mutation Causes Hyperinsulinism, Hypopituitarism With Craniofacial and Endoderm-Derived Organ Abnormalities. *Hum Mol Genet* (2017) 26:4315–26. doi: 10.1093/hmg/ddx318
103. Gregory, LC, Ferreira, CB, Young-Baird, SK, Williams, HJ, Harakalova, M, van Haaften, G, et al. Impaired EIF2S3 Function Associated With a Novel Phenotype of X-Linked Hypopituitarism With Glucose Dysregulation. *EBioMedicine* (2019) 42:470–80. doi: 10.1016/j.ebiom.2019.03.013
104. Steel, E, Hurst, JA, Cullup, T, Calder, A, Sivakumar, B, Shah, P, et al. Cenani-Lenz Syndactyly in Siblings With a Novel Homozygous LRP4 Mutation and Recurrent Hypoglycaemia. *Clin Dysmorphol* (2020) 29:73–80. doi: 10.1097/MCD.0000000000000311

105. Ocansey, S, Pullen, D, Atkinson, P, Clarke, A, Hadonou, M, Crosby, C, et al. Biallelic DNAJC3 Variants in a Neuroendocrine Developmental Disorder With Insulin Dysregulation. *Clin Dysmorphol* (2022) 31:11–7. doi: 10.1097/MCD.0000000000000397
106. Ozon, ZA, Alikasifoglu, A, Kandemir, N, Aydin, B, Gonc, EN, Karaosmanoglu, B, et al. Novel Insights Into Diabetes Mellitus Due to DNAJC3-Defect: Evolution of Neurological and Endocrine Phenotype in the Pediatric Age Group. *Pediatr Diabetes* (2020) 21:1176–82. doi: 10.1111/pedi.13098
107. Rutkowski, DT, Wu, J, Back, SH, Callaghan, MU, Ferris, SP, Iqbal, J, et al. UPR Pathways Combine to Prevent Hepatic Steatosis Caused by ER Stress-Mediated Suppression of Transcriptional Master Regulators. *Dev Cell* (2008) 15:829–40. doi: 10.1016/j.devcel.2008.10.015
108. Fazeli, W, Karakaya, M, Herkenrath, P, Vierzig, A, Dötsch, J, von Kleist-Retzow, JC, et al. Mendeliome Sequencing Enables Differential Diagnosis and Treatment of Neonatal Lactic Acidosis. *Mol Cell Pediatr* (2016) 3:22. doi: 10.1186/s40348-016-0050-x
109. Biesecker, LG. Secondary Findings in Exome Slices, Virtual Panels, and Anticipatory Sequencing. *Genet Med* (2018) 21:41–3. doi: 10.1038/s41436-018-0019-3
110. Woerner, AC, Gallagher, RC, Vockley, J, Adhikari, AN. The Use of Whole Genome and Exome Sequencing for Newborn Screening: Challenges and Opportunities for Population Health. *Front Pediatr* (2021) 9:663752. doi: 10.3389/fped.2021.663752
111. Horton, RH, Lucassen, AM. Recent Developments in Genetic/Genomic Medicine. *Clin Sci (Lond)* (2019) 133:697–708. doi: 10.1042/CS20180436
112. Mitsuhashi, S, Matsumoto, N. Long-Read Sequencing for Rare Human Genetic Diseases. *J Hum Genet* (2020) 65:11–9. doi: 10.1038/s10038-019-0671-8
113. McInerney-Leo, AM, Duncan, EL. Massively Parallel Sequencing for Rare Genetic Disorders: Potential and Pitfalls. *Front Endocrinol (Lausanne)* (2021) 11:628946. doi: 10.3389/fendo.2020.628946
114. Richards, S, Aziz, N, Bale, S, Bick, D, Das, S, Gastier-Foster, J, et al. Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* (2015) 17:405–24. doi: 10.1038/gim.2015.30
115. Ponzi, E, Alesi, V, Lepri, FR, Genovese, S, Loddo, S, Mucciolo, M, et al. Uniparental Isodisomy of Chromosome 1 Results in Glycogen Storage Disease Type III With Profound Growth Retardation. *Mol Genet Genom Med* (2019) 7:e634. doi: 10.1002/mgg3.634
116. Maguolo, A, Rodella, G, Dianin, A, Nurti, R, Monge, I, Rigotti, E, et al. Diagnosis, Genetic Characterization and Clinical Follow Up of Mitochondrial Fatty Acid Oxidation Disorders in the New Era of Expanded Newborn Screening: A Single Centre Experience. *Mol Genet Metab Rep* (2020) 24:100632. doi: 10.1016/j.ymgmr.2020.100632

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Congenital Hyperinsulinism International: A Community Focused on Improving the Lives of People Living With Congenital Hyperinsulinism

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Congenital hyperinsulinism (HI) is a rare disease affecting newborns. HI causes severe hypoglycemia due to the overproduction of insulin. The signs and symptoms of hypoglycemia in HI babies is often not discovered until brain damage has already occurred. Prolonged hypoglycemia from HI can even lead to death. Disease management is often complex with a high burden on caregivers. Treatment options are extremely limited and often require long hospital stays to devise. Cascading from suboptimal treatments and diagnostic practices are a host of other problems and challenges that many with HI and their families experience including continued fear of hypoglycemia and feeding problems. The aim of this paper is (1) to describe the current challenges of living with HI including diagnosis and disease management told from the perspective of people who live with the condition (2), to provide family stories of life with HI, and (3) to share how a rare disease patient organization, Congenital Hyperinsulinism International (CHI) is working to improve the lives of HI patients and their families. CHI is a United States based nonprofit organization with a global focus. The paper communicates the programs the patient advocacy organization has put into place to support HI families through its virtual and in-person gatherings. The organization also helps individuals access diagnostics, medical experts, and treatments. CHI also raises awareness of HI to improve patient outcomes with information about HI and prolonged hypoglycemia in twenty-three languages. CHI drives innovation for new and better treatments by funding research pilot grants, conducting research through the HI Global Registry, and providing patient experience expertise to researchers developing new treatments. The organization is also the sponsor of the CHI Collaborative Research Network which brings medical and scientific experts together for the development of a patient-focused prioritized research agenda.

Keywords: congenital hyperinsulinism, hypoglycemia, rare disease, burden of disease, challenges, patient organization

CONGENITAL HYPERINSULINISM

Congenital hyperinsulinism (HI) is a disease that causes severe hypoglycemia due to the overproduction of insulin (1–7). While the mechanism of disease depends on the subtype, in all people with HI, the close regulation of blood glucose and insulin secretion is lost. This is dangerous for newborns because it can cause an inadequate supply of fuel which is needed for growth and development (1–7). When newborns experience this lack of energy, seizures or even coma can occur. If severe hypoglycemia is prolonged, brain damage may occur which can cause permanent seizure disorders, learning disabilities, movement, vision disorders, developmental disabilities, cerebral palsy, and even death (2–10).

The worst outcomes of the disease, brain damage and death, are preventable if the baby's hypoglycemia is discovered before it is prolonged (4–6). For some, damage from hypoglycemia can occur in the first days of life. Many born with the disease are found to have hypoglycemia and diagnosed with HI in the first days, weeks, or months of life. Others are diagnosed later in the first year, and some may not be diagnosed until later in life.

There are many HI subtypes, characterized by genetic type (known or unknown), whether the condition is persistent or transient, diffuse or focal, or if the patient responds to diazoxide (1–10). Prevalence is reported to be from 1 in 2,500 births to 1 in 50,000 births, depending on where the individual with HI is born (3–6, 11–13).

Once someone is diagnosed, an individualized management plan is developed over weeks or months. Typically, the patient is hospitalized during this period. Treatment options range from simple to complex. A subset of patients are treated and well-managed on diazoxide, an oral medication discussed later in the paper because of its unwanted side effects. For those who can access genetic testing, specialized imaging, and an experienced surgeon, a cure is possible for focal disease; in this type of HI, abnormal pancreatic beta cells are limited to a focal lesion surrounded by normal beta cells. For the others with the condition, disease management is complicated, including one or more of the following: use of off-label medications *via* monthly or multiple daily injections or insulin pump, background dextrose through a feeding pump and gastrostomy tube, partial or subtotal pancreatectomy, frequent eating, and activity restrictions. No matter the type of HI, frequent daily blood glucose level checks with a home glucometer, and often with a continuous glucose monitor (CGM) are part of daily management (3–6, 14).

For many, medical management lessens over time; sometimes this occurs in a matter of months, when the condition is considered transient. For others, stabilization does not occur until later in childhood. Still others need medical management for decades or a lifetime (3–6, 8–10, 14).

In addition to the management of blood glucose to avoid hypoglycemia, people with HI often have other physical, developmental, and psychiatric issues. Feeding problems resulting from disease management and the condition itself, neurodiversity, and developmental delays resulting from prolonged hypoglycemia, and frequent co-morbidities like epilepsy, compound the issue of managing hypoglycemia on a regular basis (3–6).

Adding to the medically complex side of HI is the frequent and persistent fear parents of children with HI have that their child will experience hypoglycemia even when the child is being treated for the condition (**Table 1**). Since brain damage occurs in a large subset of those with HI, these fears have a basis in reality. Taken altogether, living with HI can be all consuming and HI families often feel the condition rules their lives (6).

CONGENITAL HYPERINSULINISM INTERNATIONAL

In 2005, with the mission to improve life for people with HI, an international group of parents of children with HI, who met through a parent email group, founded a patient advocacy organization, Congenital Hyperinsulinism International (CHI). CHI has focused its work on providing support to families, raising awareness to improve knowledge of HI among medical professionals and the general public, and contributing to research for a better understanding of the condition for more advanced diagnostics, new treatments, and cures.

The CHI Board of Directors (BOD) is the governing board for the United States (US) based nonprofit. Today, the BOD is comprised of six mothers of children born with HI. Each member has a skill or experience key to sustaining the nonprofit. The BOD has fiscal responsibility for CHI and sets its strategic direction. CHI also has a highly active advisory board that includes leading medical and scientific experts. CHI's

TABLE 1 | Challenges of Living with HI.

The challenges of living with HI depend on the following factors:
• Age of diagnosis
• Access to a multi-disciplinary team with CHI expertise
• Time spent inpatient until an acceptable individualized disease management plan is established
• Neurologic differences from prolonged hypoglycemia
• Frequency, severity, and duration of hypoglycemic events
• Difficulty feeding and eating
• Frequency of feeds
• Reliance on nasal-gastric or gastrostomy tube
• GI issues including pain, frequent vomiting, and constipation, etc.
• Presence of a syndrome or other major co-morbidities
• Availability of medication, blood sugar supplies, and continuous glucose monitoring (CGM)
• Stage of life (newborn, infant, toddler, school age child, teenager, young adult, adult, senior)
• Level of anxiety or depression of caregivers
• Resilience
• Support for caregivers and availability of home-nursing, when necessary
• Availability of quality food
• Socioeconomic level of family
• Extent to which normal activities are curtailed to maintain euglycemia or perceived threat of hypoglycemia
• Need for home-nursing care
• Childcare, quality of education and availability of educational and school support

globally focused programs are led by the CHI staff, which currently includes five full-time professionals. CHI is also well supported by a large group of volunteers.

The aim of this paper is (1) to describe the current challenges of living with HI including diagnosis and disease management told from the perspective of people living with the condition, (2) to provide family stories of life with HI, and (3) to share how a rare disease patient organization, Congenital Hyperinsulinism International (CHI) is working to improve the lives of HI patients and their families. CHI is a United States based nonprofit organization with a global focus (**Figure 1**).

HI THROUGH A PATIENT FAMILY LENS

To increase understanding of the experience of living with HI, to better understand the challenges, and as a personal counterpoint to the research and program work, which is described in the paper, three of the authors share their own family stories of diagnosis, finding treatment and care, and living and dying with HI.

Parent of a Child With Hyperinsulinism-Hyperammonemia (HI/HA)

"There is nothing that can prepare one to travel the path of parenting a child with a rare disease. My daughter, now twenty-one, was officially diagnosed with HI/HA when she was fifteen months old. The journey to diagnosis was the scariest time of my life. Seizures, failure to thrive, inpatient stays, and monitoring at several children's hospitals led to no answers. It was after these failed efforts that, as parents, we realized we had to search for answers on our own. We did and found answers across the country at the Children's Hospital of Philadelphia (CHOP).

With a diagnosis and treatment plan identified, we resumed our daily lives with a slightly redefined focus. While no parent can ever identify, with certainty, the course of daily happenings of any child, the parents of a child with a rare disease face additional obstacles that include daily and lifelong challenges, personal and professional sacrifices, and feelings of guilt watching their child suffer.

With HI/HA, the most intense challenges revolve around unstable and unpredictable changes in blood glucose levels, dietary restrictions, availability of medication used to treat HI/HA, and a lack of knowledge or understanding of the long-term impact of HI/HA itself and the medication used for treatment.

In addition to producing excessive amounts of insulin inconsistently throughout the day, the HI/HA pancreas overproduces insulin when individuals eat protein, specifically the amino acid leucine. Finding the delicate balance of appropriate fasting times and balanced nutrition consumption can be unachievable. What works sometimes, does not work other times. Any internal or external shift in normalcy also impacts blood glucose levels. This includes teething, growth spurts, fevers, illness, puberty, excessive heat, excessive cold, exercise, etc. Therefore, the art of finding the balance constantly changes, making stable glucose levels nearly impossible.

Diagnosis - More timely diagnosis to prevent brain damage and death	• HI awareness materials in twenty-three languages • Funding targeted HI genetic testing through partnership with the University of Exeter • Presenting and exhibiting at conferences and meetings • Advocating for stringent hypoglycemia neonatal guidelines
Finding appropriate multidisciplinary medical treatment for HI	• CHI Centers of Excellence Designation
Access to medications and devices used for HI	• Partnerships with Direct Relief and WEP Clinical which enable donation of medication to neediest patients • Successful application adding diazoxide to WHO List of Essential Medication • Advocating for access to continuous glucose monitoring for people with HI
Understanding the natural history of the condition and development of novel treatments, cures, and diagnostic devices	• HI Global Registry • Consulting to biotechs • Funding pilot research grants • Dissemination of clinical research opportunities • Collaborative Research Network • Hosting and participating in research conferences
Creating a lasting infrastructure for collaboration leading to innovations in clinical trial design, medical and surgical treatments, genetics, diagnostics, care guidelines, and glucose monitoring	• Collaborative Research Network
Supporting people with HI and their families	• Congenital Hyperinsulinism Support Forum • Hosting Family Conferences • Emergency funds at Centers of Excellence • Social media campaigns

FIGURE 1 | CHI Program Areas.

Proglycem (diazoxide is the generic name) truly is what keeps our daughter alive and is the one approved medication that can help reduce insulin secretion. However, it is not effective for the hyperammonemia component of HI/HA. Therefore, individuals with HI/HA must be acutely aware of protein ingestion and there may be a neurologic consequence to elevated levels of ammonia. Protein is an integral nutritional necessity. When the individual with HI/HA is a child, there can be a high level of parental control over foods eaten; however, as an adult trying to integrate into general society, controlling protein intake as a component of HI/HA management has at times been traumatic for our daughter. It has created feelings of extreme self-consciousness.

Perhaps most worrisome, for a parent of a child with HI (HI/HA), is the unintentional lack of understanding of HI/HA for our children moving from childhood into adulthood. The HI medical professionals included in our child's care are phenomenal. We owe our child's life to them. But, when questions arise such as childbearing, long-term effects of being on high doses of diazoxide, and future medical issues seemingly unexplained, even the experts cannot provide insight or guidance. The unknowing initiates another layer of concern and worry for our 21-year-old female adult who is beginning to think of what life looks like in the future. Will she be able to have children (diazoxide has a warning that it cannot be taken when pregnant), will her children have this rare disease, how has diazoxide use for more than twenty years affected her body, are questions that remain unanswered.

The ongoing challenges combined with the more recent uncertainty about what the future will look like has led to noticeable changes in my daughter's mental health and wellbeing. As parents, we prioritize continuing to seek answers, advocating for our daughter, and supporting all her medical, physical, and emotional needs that exist due to HI/HA."

Parent of a Child Born With Diffuse HI

"Despite my husband and I having prenatal genetic testing for the panel of diseases that are more common in Ashkenazi Jewish families, we did not discover we were carriers of disease-causing mutations in the ABCC8 gene until after our son was born. Our son was born 25 years ago and diagnosed in the second week of life.

His hypoglycemia symptoms were not recognized at birth or at any time while he was in the New York City birthing hospital despite my husband and I sharing our concerns about symptoms of extreme hunger, followed by lethargy and lack of interest in nursing. We were told by my obstetrician who came to the bedside in the newborn nursery, 'he is a perfectly normal baby,' and right on schedule, less than two days after giving birth, my son and I were discharged. Less than 24 hours after coming home from the birthing hospital, and less than 72 hours after being born, my son was readmitted to a New Jersey hospital, where he had numerous seizures upon arrival and his blood glucose level was so low it did not register. He was admitted to a NICU at this New Jersey hospital, and for a week the dedicated neonatologists performed many tests to find the source of persistent hypoglycemia (controlled with IV dextrose while testing to find the source). After several days, an experienced pediatric endocrinologist was consulted, and he made a clinical diagnosis of HI.

Once diagnosed, our son was transferred by ambulance to CHOP because of their expertise in treating children with HI. There he received superb treatment. After three months we finally brought him home. Throughout his life, he has continued to receive excellent care from CHOP. He continues his treatment there because there is no specialty care for adults born with HI in the US. His treatment for hypoglycemia due to HI included three subtotal pancreatectomies, background dextrose through a g-tube, and off-label use of octreotide and glucagon. He required private duty nursing at home for many years because he often needed urgent treatment for hypoglycemia day and night, even after surgeries and with medical treatment for HI (continuous glucose monitors did not yet exist). He had a brief period in his "tweens" when his blood glucose levels were in the normal range without medical treatment. At twelve years old, he became diabetic, which we knew would ultimately occur at some point because of his three subtotal pancreatectomies that removed 99% of his pancreas. The surgeries also caused permanent pancreatic insufficiency.

Prolonged hypoglycemia in the first three days of life caused my son to have irreversible disabilities. He has low vision from nystagmus and strabismus, fine motor-coordination issues, learning disabilities, and epilepsy. In the early years, life truly revolved around keeping his blood glucose levels in a safe range with medication and diet. Chronic constipation and stomach pain were often present. The combination of needing to eat to keep blood glucose levels in a safe range, to avoid additional neurological damage, combined with a lack of desire to eat, made meal and snack time fraught with anxiety for the whole family. We tried to normalize and accept the situation but there was always an underlying tension.

My son was able to attend excellent public schools in the town where we live. He was provided with accommodations, modifications, therapies, private nursing, and tutors that allowed him to learn alongside his typically developing peers. As a result of his excellent primary and secondary school education, he was able to go to college, one that supported his medical needs and learning differences. He graduated with a BA, with a concentration in education and psychology.

At 25 years old, He is trying to make the transition to independent living, which is more difficult due to his disabilities and health conditions secondary to HI. He currently lives at home and has a part-time job. He takes vigilant care of his health, is extremely focused on being physically fit, and leads a full and happy life, despite challenges. Being born with HI and having diabetes are intertwined into his identity, but they do not define him. He cares deeply about the acceptance of those who are neurodiverse and/or living with a rare disease. He has a regular practice of gratitude journaling. A caring extended family, close circle of friends, support from our local community, and close relationships with members of the CHI community have helped our son and our entire family to live a meaningful and happy life."

Parent of Children With Transient HI

"I am a parent of three children born with HI. The story of my children is rare and unique. Eleven years ago, shortly after being born, our first-born son was whisked away to the nursery because a nurse thought that he had low blood glucose. It turned out that she was right. After multiple rounds of testing on a neonate and 14-day stay in the NICU at a top hospital, we received a diagnosis of 'transient hypoglycemia.' We went home with this diagnosis, a glucometer, and a prescription for diazoxide. We were told our son needed to be fed regularly and that his little pancreas was overproducing insulin and causing his blood glucose to be low. Each day, before and after each meal, we checked our son's blood sugar, poking his tiny heels with one hand and squeezing enough with the other to put a drop of blood on the glucometer, praying that it would read above 70mg/dL. This continued for about the first four months of his life. Our first son's transient hypoglycemia was resolved by the time he was six months old.

Four years later, we welcomed our second son during a rare Nor'easter. They say that barometric pressure changes, induce labor, and I guess it was true; after 38 weeks and five days I went into labor on Valentine's Day in the wee hours of the night. Upon arrival at the hospital, we stated that we needed to check the baby's glucose when he was born; given the scenario we had with our first son, we wanted to make sure that our newborn did not have a similar issue with hypoglycemia. After many conversations with various staff including a pediatrician, we were assured that he 'did not have what your older son had' and we were released home. Going home with our newborn and this assurance made us feel ok. 36 hours later, we were back in the emergency room with a child that stopped breathing. We were told our newborn also had issues with his pancreas and an official diagnosis of HI. After learning about this diagnosis, we felt like our life was over. Was our firstborn really okay? Is this genetic? Why didn't the doctors listen to us at the birthing hospital? How in the world did this happen to us? Shortly thereafter, our son died.

During my third pregnancy I was riddled with anxiety and didn't have any comfort that I would get the appropriate care for her following birth. My daughter was born at one of the CHI Centers of Excellence at 39 weeks with a confirmed diagnosis of HI. She spent an additional week in the NICU and endocrine floor completing all the necessary testing. She too went home with a glucometer and had to be fed and checked almost every 3-4 hours. After her first 9 months, her transient hypoglycemia resolved, and she was "cured." I am happy that she got a happy ending and now is a thriving 4-year-old child. This however, does not change our incalculable loss of our second son.

Following the events of our second son's birth and subsequent death, I found the patient advocacy organization, CHI. Realizing our son's death was 100% preventable made me want to do something to ensure that other parents did not experience such a loss. Timely diagnosis of HI and treatment could have saved my child's life. Protocols that ensure neonates with a family history of low blood glucose are managed correctly need to be shared widely in the pediatric world. That is why I got involved with the advocacy organization CHI. The purpose of my involvement is to educate others by sharing our story and informing them that glucose is crucial to a newborn and that simple measures such as checking a blood glucose level could be life changing."

These are just three examples of family experiences with HI. Each family history with HI is unique.

RAISING AWARENESS TO INCREASE TIMELY DIAGNOSIS

The family accounts in the previous section show the effect of delayed diagnosis and the harm it causes. With HI, "diagnosis in a timely manner" means uncovering hypoglycemia right after birth. CHI shares the hypoglycemia guidelines published by HI specialists in 2015 (15), and advocates for these guidelines to be adopted at individual institutions, to increase the number of neonates diagnosed and properly managed before brain damage or death occur. To increase knowledge of HI and hypoglycemia in the medical and general community, CHI has created the *What is HI?* and *The Signs and Symptoms of Hypoglycemia* awareness posters (**Figure 2**).

CHI posters are now available in 23 languages: Arabic, Bulgarian, Catalan, Chinese Simplified, Chinese Traditional, Czech, Dutch, English, French, German, Georgian, Greek, Hebrew, Hungarian, Italian, Polish, Portuguese, Russian, Serbian, Slovak, Spanish, Swedish and Turkish. CHI has also created smaller 4x6 inch card sized *Say Hi to HI* infographics that can be printed and shared easily with teachers, friends and family that explain HI, hypoglycemia, and its effects in the simplest terms. The educational postcards are available in English, French, German, Russian and Spanish. With these posters and postcards available in many languages, CHI is working to ensure that knowledge of the risks associated with prolonged hypoglycemia in newborns is available globally.

GENETIC TESTING FOR PERSONALIZED CARE

Genetic testing is often a crucial element in determining what is the best treatment for each person with HI. CHI funds targeted HI genetic testing done at the University of Exeter Clinical Laboratory in the UK. This laboratory is renowned for its groundbreaking work in HI genetics. With this program, genetic testing is free of charge to families from anywhere in the world who do not have this testing covered by a healthcare plan (**Figure 3**).

Between July 2018, when the program began, and December 2021 CHI has funded genetic testing for 584 individuals from 55 different countries across five continents with medically diagnosed HI (16). An additional 460 samples were received from family members. An example of the importance of genetic testing to the diagnostic process is that it offers valuable information about the likelihood of focal disease, which can be cured. Since the program's inception, 69 patients have been found to have a single paternally inherited mutation in the ABCC8 or KCNJ11 gene which is evidence the condition may be focal in those children (16).

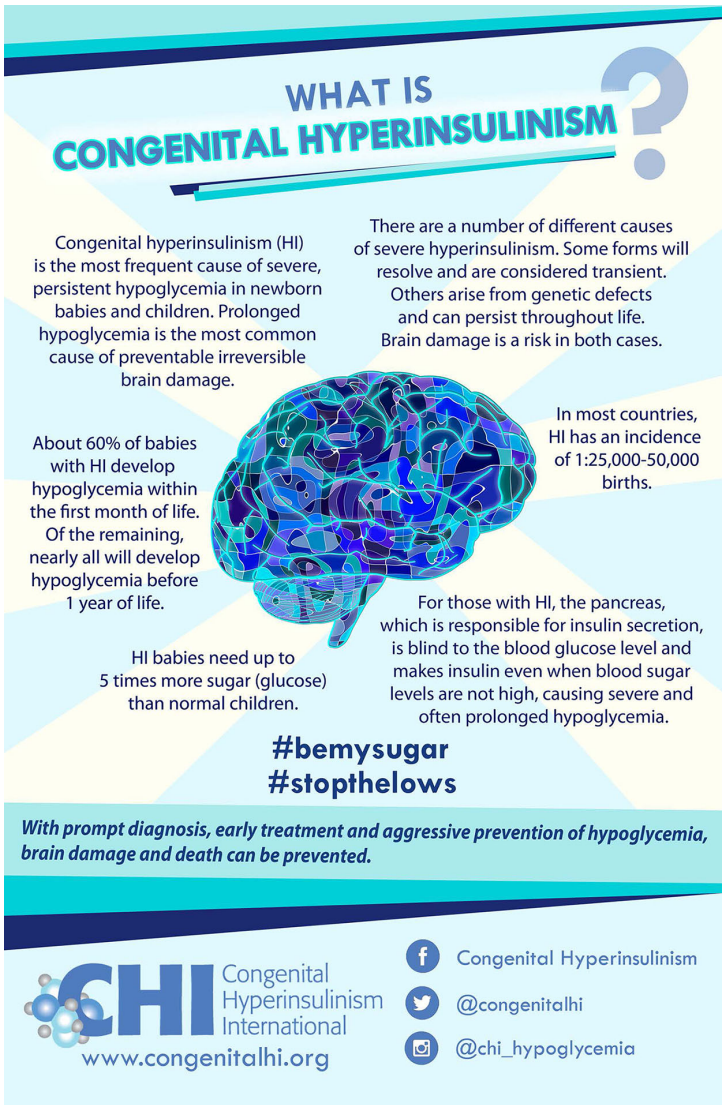

FIGURE 2 | CHI Awareness Posters.

To further share knowledge of HI and neonatal and childhood hypoglycemia and to spread knowledge of the importance of timely diagnosis, CHI also exhibits at international medical conferences and engages in social media campaigns on four social media channels.

THE SEARCH FOR EXCELLENT MULTIDISCIPLINARY MEDICAL CARE FOR HI

Once diagnosed, it is often a challenge to find medical professionals with enough knowledge of HI and a multi-disciplinary team to provide optimal treatment for it. To ensure HI families have information about where to go for comprehensive specialized HI medical care, CHI recognizes and designates expert centers, CHI Centers of Excellence (COE), that provide the highest level of multi-disciplinary care to HI patients and their families.

The CHI COE designation also recognizes an on-going commitment to research and collaboration (17). The first group of six centers was designated in 2021 (**Figure 4**). To receive the designation, centers completed an online application consisting of 33 elements including multidisciplinary expertise in fields such as surgery, gastroenterology, diet/nutrition and feeding, psychiatry, neurology, pathology, radiology, genetics, and neonatology. In addition to medical fields associated with being able to provide successful pancreatectomies, the other areas of expertise are necessary because there are often other

health issues, in addition to hypoglycemia, that HI patients need addressed (17).

Centers that have received the designation are leaders in HI research, and provide continuing medical education at the local, national, and international level. For CHI has set up special support funds to help HI families with financial needs pay for transportation, food, and hotel expenses. Presently, funds for this purpose are available to families at CHOP and Cook Children's Medical Center.

CHI also helps to facilitate international collaborations leading to standardized guidelines that support an individualized approach to care that will be supported by clinical outcomes evidence. CHI shares the patient family perspective for such projects and provides a virtual meeting place and infrastructure for collaboration.

SUPPORTING PEOPLE WITH HI AND THEIR FAMILIES

In addition to having an excellent care team, families affected by HI need ongoing support. Launched in 2011, the CHI Family Support Forum with over 1,900 members from 79 countries is a private online space where individuals affected by HI help one another cope with the stress of living with the condition and can share and receive feedback on the challenges or triumphs they experience at any given time or any topic about which they are searching for information (18).

The Forum is open to any parent of a child with HI and teenagers and adults with HI. Close family members can also join if the parents of the child with HI consent. Frequent topics include centers of excellence, feeding difficulties, child development, sharing feelings of depression or anxiety, genetics, side effects of medications, when and how to go back to work, symptoms comparisons, future pregnancies, and sharing milestones. Members understand that the forum's purpose is to support and share experiences, and not to provide unsubstantiated medical advice. Members often post photos and videos. The Forum is searchable by topic.

In addition to the private CHI Family Support Forum on Facebook, CHI maintains meaningful connections to the HI community through the social media channels Facebook, Instagram, Twitter, and LinkedIn. On these channels, CHI shares research and advocacy news, clinical research opportunities, and HI patient and family stories. Through CHI social media channels, CHI regularly reaches newly diagnosed families. CHI also maintains a highly searched webpage on HI with 17,423 visitors from 155 countries in 2021 (19).

CHI family conferences are another way families can gain support, learn, and share their experiences. CHI has organized twenty-three international conferences in the US, Europe, and virtually. Frequent agenda topics are coping with the stress of living with a rare, chronic condition, diagnostic and cure fasts, "Ask the Experts," understanding genetics, and learning about new investigational treatments. Increasingly medical professionals are joining the family conferences as participants,

FIGURE 3 | Exeter and CHI Referral Countries.

FIGURE 4 | CHI Inaugural Centers of Excellence.

to learn about HI from the medical professional and scientist speakers, as well as to gain insight into the HI patient and family perspective.

RARE DISEASE MEDICATIONS AND DEVICES CAN BE HARD TO ACCESS

For many who respond to diazoxide or a form of octreotide, there are still significant hurdles to accessing these drugs. In 2017 and 2018, CHI surveyed 74 pediatric endocrinologists from 42 countries about access issues. The survey revealed that 64% of responding pediatric endocrinologist have patients who have trouble accessing diazoxide (20).

Later in 2018, with the hope of expanding diazoxide access, CHI supported an application for diazoxide to be added to the WHO list of essential medications. The application was approved and diazoxide is now on this list (21).

CHI has also developed relationships with the manufacturers of diazoxide and other drugs used off-label but regularly prescribed by HI doctors. Through collaborations with the global nonprofit Direct Relief and WEP Clinical. CHI has been able to facilitate regular donations to the neediest patients in areas where it is most difficult to obtain the medications. There are still many HI families who have trouble accessing diazoxide, which is why CHI works to increase market access and to expand donation programs.

Another access issue for people is that home devices for measuring blood glucose levels (home glucometers and CGMs) are not made or approved for people with HI. These devices are approved by regulatory bodies for people with diabetes.

CGMs have made living with diabetes far easier, and they have also improved disease management. A CGM gives the wearer continuous information about blood glucose levels rather than just one reading in time. In this way, CGM guides treatment and helps people with diabetes have better blood glucose control. Not having to prick their finger multiple times a day to measure blood glucose is also a major improvement in the lives of people with diabetes who choose and can access CGMs.

A subset of people with CHI have been able to obtain CGM devices and supplies including 44% (n=61) of participants in the HI Global Registry (14). According to experiences shared in the patient Forum those that do typically find them useful and reasonably accurate. A smaller group of HI families have tried CGMs and chosen not to use them routinely because they do not find they track close to home glucometer values, and in some cases the wearer experiences frequent compression lows (22).

CHI advocates for individuals with HI seeking to gain access to CGMs and glucometers and supplies when there are issues with healthcare plan coverage. CHI is also encouraging CGM research that will lead to regulatory approval of CGMs for people with HI and other rare hypoglycemia disorders. Universal access to devices that are convenient and accurately measure blood glucose levels are as necessary for HI as they are for diabetes.

THE PROBLEMS WITH CURRENT TREATMENTS AND DIAGNOSIS UNCOVERED IN CHI'S HI GLOBAL REGISTRY

To accelerate the development of new treatments and cures, to increase timely diagnosis, and to improve care, and the understanding of HI, in 2018, CHI launched the HI Global Registry, a patient-reported IRB approved natural history study (**Figure 5**) (14). HIGR is hosted on the IAMRARE™ Platform which was developed by the National Organization for Rare Disorders with input from patients, caregivers, and government stakeholders to ensure a safe and user-friendly system for study participation (23). Members of a steering committee made up of leading international HI specialists and patient advocates advised on the development of the HIGR surveys and participate in future developments to strengthen it as a resource. The registry consists of a series of online surveys that ask the participant questions about the patient's experience with the disorder over his or her lifetime. This information is then aggregated to produce research reports that can be studied by researchers.

As of September 1, 2021, HIGR has participants from 46 countries and a total of 335 enrollees. Participants can complete one or more of the thirteen surveys relating to the experience of living with HI. The patient reported data shared below reinforces clinician and academic research and provides new insights patients are uniquely positioned to share.

Forty-one percent (n=29) of 70 participants with diffuse HI report hypoglycemia defined as below 70 mg/dL or 3.9 mmol several times a week or more. Participants in this group include those taking diazoxide, octreotide, and lanreotide.

HIGR also collects data related to medication side-effects including an increase in body hair in 85% (n=93) of 110 participants taking diazoxide. This physical alteration can have a profound psychosocial impact on individuals, especially children.

In addition to on-going hypoglycemia and medication side-effects many HIGR respondents report other diagnoses or symptoms that impact their daily life. Of 124 respondents, 38% (n=47) report participants have a chronic neurologic problem which they feel is due to the participant's prolonged hypoglycemia and 44% (n=57) report delays in meeting developmental milestones. Of 132 respondents, feeding issues were experienced by 69% (n=91) of participants with 40% (n=53) experiencing issues with appetite, and 39% (n=52) refusing to eat. The lack of desire to eat and the risks of not eating for those with HI produces a cycle of anxiety for both the HI child and caregivers. Thirteen percent (n=18) of 135 individuals who responded to the Other Diagnoses Survey report the participant has epilepsy. The frequency with which respondents report neurologic developmental issues reinforces the need for discovering and treating hypoglycemia before brain damage occurs.

The physical and psychological health of parents of children with HI are impacted by the stress of trying to maintain normal blood glucose and treatments that interfere with daily life. Parents of 48% (n=59) of the 123 participants report their physical health has suffered from having a child with a HI-related condition. Parents of 67% (n=83) of the 123 participants report their mental health has suffered from having a child with a HI-related condition. Individuals are included in this statistic if they answered that their health suffered "somewhat," "quite a lot," or "very much."

Individuals who undergo a pancreatectomy are often trading one disease for another. All 9 people who report having diabetes had a pancreatectomy and five take pancreatic enzymes for pancreatic insufficiency. The other 12 individuals who underwent a subtotal pancreatectomy and do not report developing diabetes are under the age of 13. This shows the need for a treatment that would reverse diabetes and pancreatic insufficiency for those who have undergone subtotal pancreatectomy for HI.

Since HIGR was launched, 13 participants with a mutation in the GLUD 1 gene that causes HI/HA have been reported. People with HI/HA have hypoglycemia due to glutamate dehydrogenase (GDH) over-activity in the pancreatic beta cells, which can be treated with diazoxide. However, these patients also have other medical problems that may be due to GDH over-activity in other cell types causing seizures and developmental delays. These patients need a treatment to address the neurologic issues that are not caused by prolonged hypoglycemia.

As a rare disease patient organization, it is critical that CHI helps drive a patient-centric research strategy. Recruitment for the registry is on-going as additional patient participation can inform avenues for future research studies, identify areas of patient needs, and improve clinical care. It is especially important to detail the natural history for sub-group analysis of HI patients. Therefore, we encourage all individuals living with HI or their caregivers to join the registry and for clinicians to encourage their patients to participate.

THE PROMISE OF INVESTIGATIONAL TREATMENTS

Beginning in 2015 there has been a significant increase in HI research in pre-clinical and clinical phases with the potential to lead to new treatments (24). These important research projects are taking place at academic institutions and commercial biotechnology companies. CHI supports four of these research studies by providing data from HIGR to help the researchers develop innovative and patient-informed clinical trial design.

CHI also supports clinical development programs by sharing the patient journey through presentations, discussions, and listening sessions. CHI also reviews protocols and informed consent forms. In addition, CHI informs the patient community of the importance of clinical research for the development of better diagnostics, new treatments, and cures

To further support the development of new treatments, better diagnostic tools and practices, and an improved quality of life, CHI has supported eight pilot research grants, seven through the Million Dollar Bike Ride (**Table 2**). The Million Dollar Bike Ride is a project of the Orphan Disease Center (ODC) at the University of Pennsylvania. With an annual bike ride in Philadelphia as a fundraising vehicle, dozens of rare disease teams raise funds for rare disease research. Each team determines a disease-focused research topic and submits language to the ODC for the Request for Applications. Each team provides a roster of potential reviewers with expert knowledge of the disease area. The ODC oversees the selection process, administers the grants, and matches a sizable portion of the funds raised (25). CHI has also administered one grant directly, the hyperinsulinism hyperammonemia pilot grant (26).

A LASTING INFRASTRUCTURE FOR COLLABORATION TO ENSURE FUTURE GAINS FOR THE HI COMMUNITY

To ensure a continuing cycle of innovation, and to accelerate and help advance current research projects, CHI has launched a collaborative research network (CRN) with 56 council members. Council members are researchers, clinicians, and patient leaders from 19 countries, 21 hospitals and academic institutions, five biotech companies, and three nonprofits that support people with CHI. Retired clinicians and researchers who paved the way for today's progress and patients and caregiver leaders round out the team.

The CHI CRN is led by a core team consisting of a lead researcher and clinician and three CHI staff members. Council members have joined one of seven workstreams: *Care Guidelines/*

FIGURE 5 | HI Global Registry Banner Image.

TABLE 2 | Million Dollar Bike Ride and CHI International Pilot Grants.

Million Dollar Bike Ride Grants	
Year	**Description**
2014	Diva De Leon Crutchlow, MD, of the Children's Hospital of Pennsylvania, was awarded $60,000 for a pilot study investigating the efficacy and safety of sirolimus in the treatment of congenital hyperinsulinism. This research was very important at the time because sirolimus was being prescribed off-label for patients with congenital hyperinsulinism, and there was a lack of research in this population.
2015	Mark Dunne, PhD, of the University of Manchester, received $71,000 for the topic: Toward Precision Medicine in the Treatment of Congenital Hyperinsulinism in Infancy. The focus was on expanding islet cell study to include not only the study of beta cells. This research is very important because current medications are often poorly tolerated, ineffective, with adverse effects.
2016	Changhong Li, MD, PhD, then at the Children's Hospital of Philadelphia, was awarded $82,000 for Drug Development for treatment of Glutamate Dehydrogenase Hyperinsulinism. The goal of this study was to identify a safe medicine that will treat all the health issues resulting from activating mutations in GDH in people with GDH-HI. Dr. Li continues to research new treatments for GDH-HI as the Associate Director at Nanjing Institute of Advanced Biotechnology.
2017	Diva De Leon Crutchlow, MD, of the Children's Hospital of Pennsylvania, received $87,109 to research the Bihormonal Bionic Pancreas for the treatment of Diabetes Post-Pancreatectomy in Children with Congenital Hyperinsulinism. She collaborated with Dr. Steven Russell of MGH and Dr. Ed Damiano of Boston University and Beta Bionics to examine the safety and efficacy of the Bionic Pancreas system in children and young adults with hyperinsulinism who have developed diabetes after pancreatectomy.
2018	Amanda Ackermann, MD, PhD, of the Children's Hospital of Philadelphia, was the grantee. She received $84,080 for research on Vitamin E Supplementation in Hyperinsulinism/Hyperammonemia Syndrome. Vitamin E has been tested in human cell lines and mice with activating GDH mutations and shows potential promise as a treatment for those with GDH HI (HI/HA). The study looks to see if vitamin E supplementation is well tolerated and reduces hypoglycemia, hyperammonemia, and seizures.
2019	Thomas Smith, PhD, of the University of Texas, Medical Branch, received a grant of $72,014. Once again, the focus of this grant was "Towards new therapeutics treatment for hyperinsulinism/hyperammonemia syndrome (HI/HA). The focus here is on targeting GDH directly to treat all symptoms associated with HI/HA throughout the body.
2020	Indi Banerjee, MD, of the University of Manchester in the UK, received $73,190 for the research topic "Maximizing the utilization of the Hyperinsulinism Global Registry (HIGR)." His proposed study which includes partners from a number of institutions in Europe, the US, and Asia aims to "build on the opportunity to add medical grade information to existing parent reported HIGR information, thereby joining up clinical and parent perspectives in the search towards better understanding and improved treatment for HI. MaxHIGR will lay the basis for HIGR to evolve into a registry that will tell us about the natural history of disease, which treatments are better and have less side effects and how we can improve the quality of life of children and families living with HI."
2021	Elizabeth Rosenfeld, MD, of Children's Hospital, received $73,045 for a study on the "Natural History of the Hyperinsulinism Hyperammonemia Syndrome – A Multi-center Observational Study Incorporating Patient-centered Data through the HI Global Registry." In the study, she will describe the natural history of the hyperinsulinism hyperammonemia syndrome using a composite approach that combines database and medical record reviews from US Congenital Hyperinsulinism Centers of Excellence, with telephone interviews, and HI Global Registry data.
CHI International Pilot Grants	
2019	CHI directly granted Amanda Ackermann, MD, PhD, of the Children's Hospital of Philadelphia funds to study a "Novel Mouse Model to Investigate Pathophysiology of Hyperinsulinism/Hyperammonemia Syndrome." Hyperinsulinism/hyperammonemia (HI/HA) syndrome is not only a disease of hypoglycemia. Patients with HI/HA syndrome also have high blood ammonia levels, seizures, and neurodevelopmental differences that currently are not well-understood and do not have any specific treatments. It has been difficult to study each of these features of HI/HA syndrome in patients because each one can affect the other features.

TABLE 3 | CHI CRN Workstreams with Mission Statements.

Workstream	Mission Statement
Care Guidelines/ Centers of Excellence	To create a prioritized research agenda on the topic of care guidelines/Centers of Excellence, we envision a better future for those with CHI through improved care guidelines, centers of excellence, and collaboration for better quality of life and outcomes for patients.
Clinical Trials/ Industry Engagement	To create a space where patient and industry leaders and academic researchers and clinicians can come together to consider collaborations and approaches to enable progress in clinical research for today's projects and tomorrow's innovations.
Diagnostics	To create a prioritized research agenda for the topic of diagnostics, we envision a better future for those with CHI through improved diagnostics for better quality of life and outcomes for patients.
Genetics	To create a prioritized research agenda for the topic of genetics, we envision a better future for those with CHI through improved understanding of genetics for better quality of life and outcomes for patients.
Glucose Monitoring	To create a prioritized research agenda for the topic of glucose monitoring, we envision a better future for those with CHI through improved glucose monitoring for better quality of life, diagnostics, and outcomes for patients.
Medical and Surgical Treatments	To create a prioritized research agenda for a better future for those with CHI through new and better medical and surgical treatments or cures.
What is HI: Nomenclature and Inclusion	To develop a plan to bring synergy to the way the patients, physicians, and medical industry decision makers describe the disease, to better define who is counted in the "CHI patient community," to agree upon a set of terms that define the condition and its subtypes, and to educate all appropriate stakeholders.

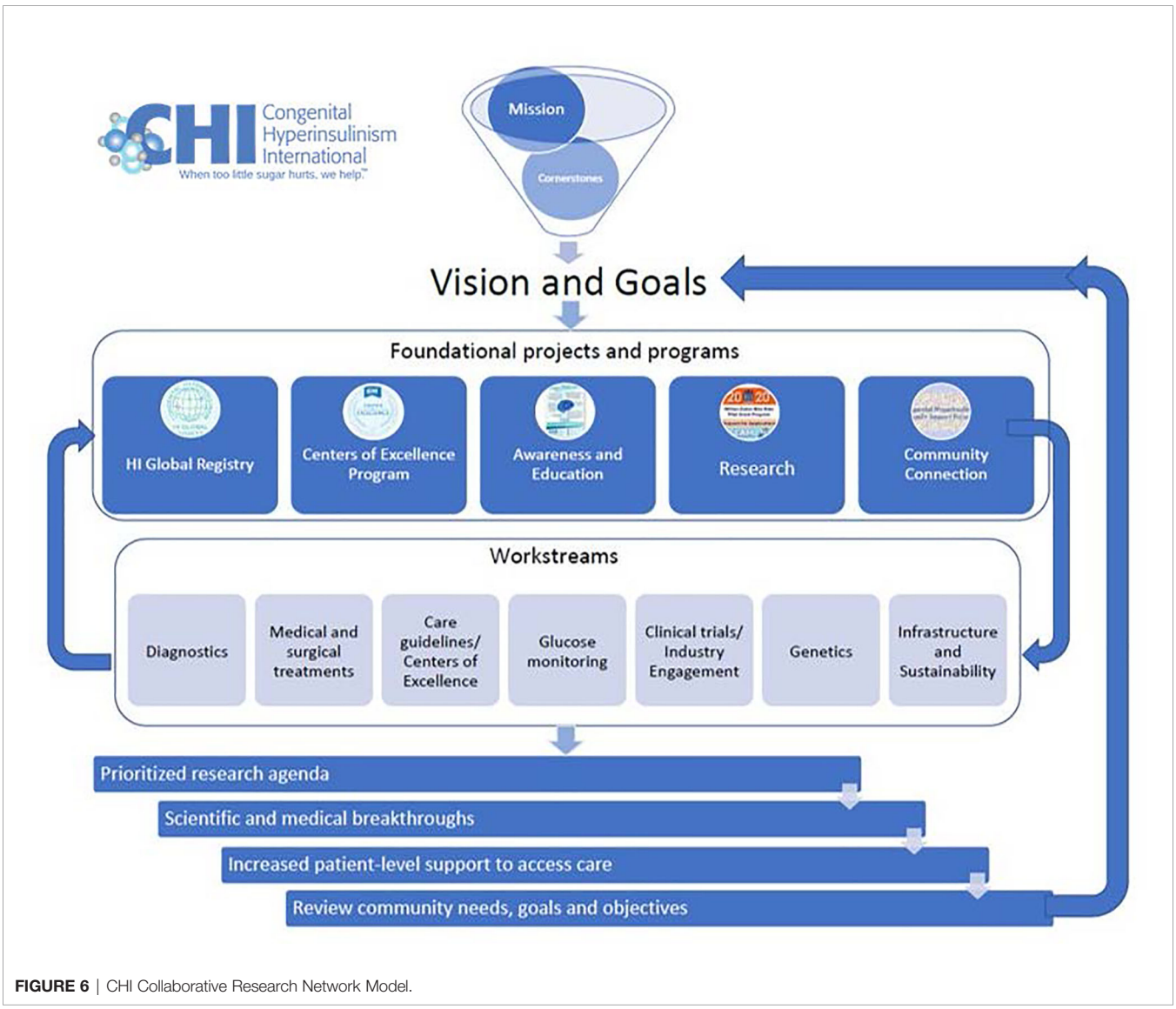

FIGURE 6 | CHI Collaborative Research Network Model.

Centers of Excellence, Clinical Trials/Industry Engagement, Diagnostics, Genetics, Glucose Monitoring, Medical and Surgical Treatments, and What is HI: Nomenclature and Inclusion (**Table 3**). Together, CRN council members are developing a patient-focused prioritized research agenda that will potentially lead to faster and more accurate diagnosis, drive new evidence-based treatments and cures, standardize clinical guidelines, and facilitate improved access to treatment, medication, and supplies (19).

The CHI CRN is made possible by the Chan Zuckerberg Initiative (CZI) Rare As One Network (RAO). In 2019, CHI applied and was accepted to be one of thirty CZI RAO patient advocacy organizations in the US (27). The RAO Network, which has now expanded to 50 patient organizations, is providing CHI with the knowledge to continue to develop and sustain our patient-led research initiative. Through RAO, CHI studied the work of the Castleman Disease Research Network (CDRN). The CDRN identified the limitations of a traditional "Request for Proposal (RFA)" approach to selecting research to be funded (28). The RFA method relies on a small group of people raising funds and selecting topics for funding. Often, a limited group of researchers in the field respond to the request, and the topic is determined by chance. In contrast, with the CRN approach, experts from a variety of institutions work collaboratively and deliberately, in a calculated manner to determine what research is necessary and to set priorities as one research community, focused on the needs of the patients and their families. CHI has adopted this approach and is refining it as needed for the HI Community.

With the initial prioritized research agenda to be completed in 2022, CHI will work with its partners to create a lasting infrastructure for collaboration, with platforms for data sharing, and consistent small and large group meetings, to actualize the research projects. CHI will use the prioritized research agenda as compass and guide for its programmatic work which will continually evolve with the progress made because of CRN research breakthroughs (**Figure 6**).

CONCLUSION

People with HI and their families need ongoing support and new and better treatments. CHI, with its many partners and supporters, collaborates with the world's leading HI experts, medical professionals, technologists, and people who live with HI and their families worldwide, to make the present as livable as possible. With financial support from individual donors, foundations, and companies CHI offers extensive research, support, and awareness programs. Together with its partners CHI is creating a brighter future for those with HI, with more timely diagnosis, more access to existing treatments, the development of new and better tools and treatments, even cures, and more support to people with HI and their families. As the CHI slogan states, "When too little sugar hurts, we help."

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by North Star Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JR, SB, DT, and JS wrote the initial draft of the article. TP provided the data analysis for the HIGR section and TP and JS provided comments, critical revisions, and support with formatting. JS provided the artwork and charts. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Stanley CA. Hyperinsulinism in Infants and Children. *Pediatr Clin North Am* (1997) 44(2):363–74. doi: 10.1016/S0031-3955(05)70481-8
2. Stanley CA. Perspective on the Genetics and Diagnosis of Congenital Hyperinsulinism Disorders. *J Clin Endocrinol Metab* (2016) 101(3):815–26. doi: 10.1210/jc.2015-3651
3. Arnoux J-B, Verkarre V, Saint-Martin C, Montravers F, Brassier A, Valayannopoulos V, et al. Congenital Hyperinsulinism: Current Trends in Diagnosis and Therapy. *Orphanet J Rare Dis* (2011) 6:63. doi: 10.1186/1750-1172-6-63
4. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ. Therapies and Outcomes of Congenital Hyperinsulinism-Induced Hypoglycaemia. *Diabetes Med J Br Diabetes Assoc* (2019) 36(1):9–21. doi: 10.1111/dme.13823
5. Rosenfeld E, Ganguly A, De Leon DD. Congenital Hyperinsulinism Disorders: Genetic and Clinical Characteristics. *Am J Med Genet C Semin Med Genet* (2019) 181(4):682–92. doi: 10.1002/ajmg.c.31737
6. Banerjee I, Raskin J, Arnoux J-B, De Leon DD, Weinzimer SA, Hammer M, et al. Congenital Hyperinsulinism in Infancy and Childhood: Challenges, Unmet Needs and the Perspective of Patients and Families. *Orphanet J Rare Dis* (2022) 17(1):61. doi: 10.1186/s13023-022-02214-y
7. Dunne MJ, Cosgrove KE, Shepherd RM, Aynsley-Green A, Lindley KJ. Hyperinsulinism in Infancy: From Basic Science to Clinical Disease. *Physiol Rev* (2004) 84(1):239–75. doi: 10.1152/physrev.00022.2003
8. Meissner T, Wendel U, Burgard P, Schaetzle S, Mayatepek E. Long-Term Follow-Up of 114 Patients With Congenital Hyperinsulinism. *Eur J Endocrinol* (2003) 149(1):43–51. doi: 10.1530/eje.0.1490043
9. Lord K, Radcliffe J, Gallagher PR, Adzick NS, Stanley CA, De León DD. High Risk of Diabetes and Neurobehavioral Deficits in Individuals With Surgically Treated Hyperinsulinism. *J Clin Endocrinol Metab* (2015) 100(11):4133–9. doi: 10.1210/jc.2015-2539
10. Menni F, de Lonlay P, Sevin C, Touati G, Peigné C, Barbier V, et al. Neurologic Outcomes of 90 Neonates and Infants With Persistent Hyperinsulinemic Hypoglycemia. *Pediatrics* (2001) 107(3):476–9. doi: 10.1542/peds.107.3.476
11. Bruining GJ. Recent Advances in Hyperinsulinism and the Pathogenesis of Diabetes Mellitus. *Curr Opin Pediatr* (1990) 2(4):758–65. doi: 10.1097/00008480-199008000-00024
12. Mathew PM, Young JM, Abu-Osba YK, Mulhern BD, Hammoudi S, Hamdan JA, et al. Persistent Neonatal Hyperinsulinism. *Clin Pediatr (Phila)* (1988) 27(3):148–51. doi: 10.1177/000992288802700307
13. Glaser B, Blech I, Krakinovsky Y, Ekstein J, Gillis D, Mazor-Aronovitch K, et al. ABCC8 Mutation Allele Frequency in the Ashkenazi Jewish Population and Risk of Focal Hyperinsulinemic Hypoglycemia. *Genet Med Off J Am Coll Med Genet* (2011) 13(10):891–4. doi: 10.1097/GIM.0b013e31821fea33
14. Hyperinsulinism Global Registry. Glen Ridge, (NJ: Congenital Hyperinsulinism International. Available at: https://www.higlobalregistry.org (Accessed 22 Jan 2022).
15. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al. Recommendations From the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. *J Pediatr* (2015) 167(2):238–45. doi: 10.1016/j.jpeds.2015.03.057
16. Raskin J, Pasquini T, Houghton J, Mesfin M, Flanagan S. Genetic Testing Access and Results for Patients With Congenital Hyperinsulinism as Conducted Through the CHI and University of Exeter Partnership. In: *Poster presented at: European Society for Pediatric Endocrinology Meeting*. (Accessed 2021 September 22-26) Virtual.
17. The CHI Centers of Excellence (COE) Program. (2021). Congenital Hyperinsulinism International. Available at: https://congenitalhi.org/wp-content/uploads/2021/07/COE-Press-Release.pdf (Accessed 2022 Feb 1).
18. Congenital Hyperinsulinism International Family Support Forum on Facebook. Available at: https://www.facebook.com/groups/hyperinsulinismsupport/ (Accessed 2022 January 23).
19. Congenital Hyperinsulinism International. Congenital Hyperinsulinism International. Available at: www.congenitalhi.org (Accessed cited 2022 Feb 1).
20. Raskin J, Pasquini T, Eaves Hood D. *Assessing Global Access to Treatments and Care for People Living With Hyperinsulinism Poster Presented at: National Organization of Rare Disorders Summit* (Accessed 2020 October 8-9). Virtual.
21. von Oettingen J, Armstrong K, Raza J, Raskin J, Zacharin M, JP C. *Application for Inclusion of Diazoxide in the WHO Model List of Essential Medicines for Children* (2019). Available at: https://www.who.int/selection_medicines/committees/expert/22/applications/s18_diazoxide.pdf (Accessed 20 Jan 2022).
22. Rayannavar A, Elci OU, Mitteer L, De Leon DD. Continuous Glucose Monitoring Systems: Are They Useful for Evaluating Glycemic Control in Children With Hyperinsulinism? *Horm Res Paediatr* (2019) 92(5):319–27. doi: 10.1159/000506230
23. IAMRARE® Registry Program. *National Organization for Rare Disorders*. Available at: https://rarediseases.org/iamrare-registry-program/ (Accessed 1 Feb 2022).
24. Home -ClinicalTrials.gov. *Search of: Congenital Hyperinsulinism on ClinicalTrials.Gov*. Available at: https://clinicaltrials.gov (Accessed cited 2022 Jan 1).
25. *Million Dollar Bike Ride*. Available at: https://www.milliondollarbikeride.org/.
26. HIHA Grant. Congenital Hyperinsulinism International. Available at: https://congenitalhi.org/hiha-grant/ (Accessed 2022 January 15).
27. Rare as One Project. *Chan Zuckerberg Initiative*. Available at: https://chanzuckerberg.com/science/programs-resources/rare-as-one/ (Accessed 1 Feb 2022).
28. Zuccato M, Shilling D, Fajgenbaum DC. The Collaborative Network Approach: A Model for Advancing Patient-Centric Research for Castleman Disease and Other Rare Diseases. *Emerg Top Life Sci* (2019) 3(1):97–105. doi: 10.1042/ETLS20180178

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Global Registries in Congenital Hyperinsulinism

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Congenital hyperinsulinism (HI) is the most frequent cause of severe, persistent hypoglycemia in newborn babies and children. There are many areas of need for HI research. Some of the most critical needs include describing the natural history of the disease, research leading to new and better treatments, and identifying and managing hypoglycemia before it is prolonged and causes brain damage or death. Patient-reported data provides a basis for understanding the day-to-day experience of living with HI. Commonly identified goals of registries include performing natural history studies, establishing a network for future product and treatment studies, and supporting patients and families to offer more successful and coordinated care. Congenital Hyperinsulinism International (CHI) created the HI Global Registry (HIGR) in October 2018 as the first global patient-powered hyperinsulinism registry. The registry consists of thirteen surveys made up of questions about the patient's experience with HI over their lifetime. An international team of HI experts, including family members of children with HI, advocates, clinicians, and researchers, developed the survey questions. HIGR is managed by CHI and advised by internationally recognized HI patient advocates and experts. This paper aims to characterize HI through the experience of individuals who live with it. This paper includes descriptive statistics on the birthing experience, hospitalizations, medication management, feeding challenges, experiences with glucose monitoring devices, and the overall disease burden to provide insights into the current data in HIGR and demonstrate the potential areas of future research. As of January 2022, 344 respondents from 37 countries consented to participate in HIGR. Parents or guardians of individuals living with HI represented 83.9% of the respondents, 15.3% were individuals living with HI. Data from HIGR has already provided insight into access challenges, patients' and caregivers' quality of life, and to inform clinical trial research programs. Data is also available to researchers seeking to study the pathophysiology of HI retrospectively or to design prospective trials related to improving HI patient outcomes. Understanding the natural history of the disease can also guide standards of care. The data generated through HIGR provides an opportunity to improve the lives of all those affected by HI.

Keywords: rare disease, registry, congenital hyperinsulinism, hypoglycemia, patient-reported outcomes

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INTRODUCTION

Congenital hyperinsulinism (HI) is the most frequent cause of severe, persistent hypoglycemia in newborn babies and children (1–3). In most countries, HI occurs in approximately 1/25,000 to 1/50,000 births (4). About 60% of babies with HI develop hypoglycemia during the first month of life. When HI is not recognized and diagnosed or if treatment is ineffective in preventing hypoglycemia, brain damage or death can occur (1–4). Although some aspects of HI are well established, many questions remain unanswered. In rare diseases, registries provide an opportunity to investigate the many unanswered research questions and help identify individuals who may be interested in future study opportunities (5–7). Congenital Hyperinsulinism International (CHI) created the HI Global Registry (HIGR) in October 2018 to collect patient-powered HI insights. For HI, HIGR provides the foundation for new research areas into the natural history and burden of the disease.

BACKGROUND

In its simplest form, a registry is a tool to collect data. Commonly identified goals include formalizing a contact list, performing natural history studies, establishing a network for future product and treatment studies, and supporting patients to offer more successful and coordinated care (5–8).

Patient-reported outcomes refer to data elements reported by patients or their representatives (6, 9, 10). Regulators have signaled an increased acceptance of the use of real-world data and patient-reported outcomes as part of clinical trial designs and regulatory decision-making (11, 12). Members of the rare disease community focus on increasing trust, especially among regulators, in the validity and use of patient-reported data (11). The use and acceptance of registries for generating data are growing, and for some diseases, patient registries may provide the most current, relevant, and in-depth research for the condition.

Patients and caregivers are uniquely positioned to provide information related to their symptoms, functional status, quality of life, and the overall burden of disease (5). Skepticism related to patient-generated data quality has subsided in recent years as rigor has increased through implementing best practices in governance, design, and data quality (13–15). Very little is known about most rare diseases, and registries can be a critical tool in providing additional insight into the medical and day-to-day experience of living with a rare disease.

KNOWLEDGE GAPS IN HI

In 2020, CHI launched the Collaborative Research Network (CRN) to establish a global network of HI experts to create a patient-focused prioritized research agenda and to formalize an infrastructure for collaboration to conduct HI research. The goals of the CRN include improving the time and accuracy of a diagnosis, driving new evidence-based treatments and cures, standardizing clinical guidelines, and facilitating improved access to treatments. The CHI CRN Council includes 56 academic and biotech researchers, clinicians, and patient leaders from 19 countries.

Through the process of building the prioritized research agenda, some of the significant gaps identified included understanding the effectiveness of current treatment options, data to support the evolving standards of care for HI patients, determining global prevalence, more information on associated comorbidities of HI, and to better understand the role of timely diagnosis on developmental outcomes. This international consortium identified HIGR as an important tool for generating new insights into the natural history of HI, medication side effects, diet and feeding habits, glucose levels, the patient experience and quality of life, and the overall burden of disease.

METHODS

The HI Global Registry (HIGR) was launched in October 2018 by Congenital Hyperinsulinism International (CHI), a nonprofit patient advocacy organization dedicated to research, awareness, and support. CHI is responsible for the day-to-day data collection, recruitment, strategic direction, and stewardship of HIGR, the only global patient-powered HI registry. The registry utilizes the IAMRARE™ online platform hosted by the National Organization for Rare Disorders and developed with input from FDA, NIH, patients, organizations, and experts in the field.

The registry is guided by a research protocol approved by an institutional review board (IRB) and advised by internationally recognized HI patient advocates and experts, known as the HI Global Registry Steering Committee. Privacy management and data security principles are followed to protect the personal data of all participants. The goal of HIGR is to advance the global understanding of HI and drive research toward better treatments and, ultimately a cure.

The primary objectives of HIGR are:

- To provide a convenient online platform for participants (or caregivers) to self-report cases of HI in order to document the natural history and outcomes of individuals with HI.
- To improve knowledge of global prevalence of HI and any associated comorbidities.
- To better understand the role of timely diagnosis of HI on patient developmental outcomes.
- To better understand patient health outcomes of different HI treatment options, settings, and provider types.
- To identify both positive and negative effects related to different HI treatment options.
- To support the evolving standards of care for HI patients using natural history and outcome information from a global perspective.

The secondary objectives of HIGR are:

- To document the obstacles to accessing HI care, supplies, and medications.
- To measure the impact of HI and its management on patients' and caregivers' quality of life.
- To aid CHI and/or other country or region-specific HI patient organizations in identifying like genotypes or similar conditions to further connect HI patients/families within the larger HI community.
- To accelerate and facilitate HI clinical study development by identifying eligible research participants quickly and efficiently.
- To serve as an aggregated, de-identified resource to researchers seeking to study the pathophysiology of HI retrospectively in order to design prospective trials related to improving HI patient outcomes.
- To support the work of the CHI Collaborative Research Network (CRN) by providing natural history data and providing a platform for future research studies.

Individuals must consent to participate in the registry. The consent process can be completed by 1) an individual 18 years or older who is diagnosed with HI or suspected of having HI or 2) a parent or recognized legal guardian of a participant who is under the age of 18 or 18 years or older with assigned guardianship. The consented individual who completes the survey is referred to as the respondent. The study participant living with HI is referred to as the participant; in some cases, the respondent can also be the study participant. When a minor reaches the age of 18, that participant is asked to complete the adult consent process to authorize continuing access.

Investigators established a set of questions across surveys with predictable, congruent responses for any participant with an HI diagnosis. Investigators may identify participants whose responses are incongruent with the diagnosis and may need closer scrutiny. Potential outcomes of this type of review include outreach to the participant to clarify answers, exclusion from specific reports, or exclusion from the project.

Respondents provide consent for the use of de-identified data in research and communication preferences. Respondents can choose to be contacted by HIGR staff for networking opportunities and to learn about research studies. Since 2019, HIGR investigators have published an annual report to the community to share key highlights from the registry. These reports are a way to provide summaries of responses from critical surveys and allow participants to see how their responses match others in the community. Data is available to investigator-approved third-party researchers seeking to study the pathophysiology of HI retrospectively or to design prospective trials related to improving HI patient outcomes. Finally, de-identified information is shared with other large-scale health initiatives, such as the Rare Disease Cures Accelerator-Data and Analytics Platform, an FDA-funded initiative hosted by NORD and the Critical Path Institute (16). Individuals may withdraw their consent at any time.

The registry consists of thirteen surveys made up of questions about the patient's experience with HI over their lifetime. An international team of HI experts, including family members of children with HI, advocates, clinicians, and researchers, developed the survey questions. The surveys are Contact Information, Demographics, Pregnancy, Birth, Diagnosis, Medication Management, Diet and Feeding Management, Surgical Management, Other Diagnoses, Glucose Monitoring Management, Development, Parent Quality of Life, and Patient Quality of Life. All surveys, except for Pregnancy and Birth, may be updated by the participant at their discretion when there is a notable change in their status, such as a new address, a change in treatment, or a newly diagnosed health condition. Respondents are asked to complete the longitudinal surveys, Glucose Monitoring Management and Quality of Life, every six months to provide information on the natural progression of the patient experience.

The registry team increases the accuracy and reliability of the registry data by sending respondents reminder emails to encourage them to update data and complete longitudinal surveys. HIGR investigators and staff monitor the data quality through automated and manual mechanisms. If the registry team determines one or more response(s) to be potentially invalid, an authorized staff member will contact the participant to clarify the response(s). The MaxHIGR sub-study, discussed below, will help confirm the overall validity and reliability of the data through the inclusion of physician generated data.

This paper shares trends observed through collected HIGR data from October 2018 to January 15, 2022. When answering surveys, individuals can answer as many or as few questions as they would like; therefore, the number of responses to questions may vary even within sections. If a question is updated or longitudinal, the most recent survey responses are reported, and descriptive statistics are provided.

RESULTS

Participants

As of January 2022, 344 individuals consented to participate in HIGR, and 248 active respondents submitted a total of 2,192 surveys. Participants ranged from 2 weeks to 52 years old. Parents or guardians of individuals living with HI represented 83.9% of the individuals completing the surveys, 15.3% were individuals living with HI, and.8% identified as another category, including spouse and Aunt/Uncle. Individuals represented 46 different countries with 60.8% from North America, 23.8% from Europe, 6.7% from Asia, 4.1% from South America, 3.8% from Australia, and.9% from Africa.

Subtype

Survey options for HI type include unknown, diffuse, focal, atypical, HI is suspected no formal diagnosis, and undiagnosed form of HI. A total of 171 individuals reported HI type. Diffuse HI is a general term that includes several forms of HI that affect the

entire pancreas; 52.1% reported diffuse disease; 10.5% reported focal HI; 4.7% reported receiving an atypical HI diagnosis. Another 32.7% report one of the unspecified responses available, including HI suspected, unknown type, and undiagnosed form; the HIGR investigators further analyzed these individuals to confirm that they had HI based on positive genetic results or reported methods of medical management (**Table 1**).

Genetics and Syndromes

Of the 146 respondents who reported HI genetic testing, 69.2% of participants had positive results for a gene associated with HI, and 61.0% (n=89) had positive genetics on the first test. An additional 33 individuals had a second test and 36.4% (n=12) had positive results. Of the 129 respondents who provided HI type and genetic testing results, 66.7% of participants tested positive for a change/mutation in HI-related genes. The type of HI included 61.6% (n=53) diffuse disease. ABCC8 was the most commonly detected gene in 45.7% (n=59) of all participants who had genetic testing (**Table 2**).

Eleven participants had a HI-related syndrome. The syndromes include Beckwith-Weidemann, Kabuki, Turner, Sotos, Fanconi, Polycystic Kidney Disease, and Rubinstein-Taybi Type 2.

Birth

Of newborns born with HI, 69.8% (n=90) were born term (37-42 weeks) with a mean weight of 3.75 kilogram (kg), 17.1% (n=22) were born between 34-36 weeks with a mean weight of 3.50 kg, and 12.4% (n=16) were born between 28-33 weeks with a mean weight of 2.46 kg (**Table 3**).

Of 170 newborns, 60% (n=102) had an abnormal blood glucose test recorded before leaving the birthing facility or being released into parental care, and 77.5% of them (n=79) reported abnormal blood glucose at one day old and 12.7% (n=13) reported abnormal blood glucose on day two. Of babies who had an abnormal blood glucose test, 50.0% (n=39) were in the normal weight range (**Table 4**). Of the newborns whose parents were aware of a blood glucose test, the age (in days) that the newborn first presented abnormal blood glucose was day one for 41.6% (n=79) of participants and day two for 6.8% (n=13).

Symptoms of hypoglycemia were first noticed for 22.0% (n=31) of all participants within the first hour of birth and an additional 18.4% (n=26) within 24 hours of birth (**Table 5**). Only 19.4% (n=33) of people reported having a fasting study before leaving the birthing facility.

No known risk factors of hypoglycemia were identified or reported shortly after delivery and before leaving the birthing facility for 28.4% (n=48) of newborns (**Table 6**). The risk factors included family history, hypoglycemia, and physical features. Only 4.7% reported a known family history of a genetic form of hypoglycemia.

Before receiving a diagnosis of HI, 91.73% (n=132) of newborns were hospitalized for symptoms, including 33.9% (n=43) who were readmitted to the hospital following their initial stay at birth. The reported length of hospitalizations ranged from less than a week (9.5%) to 11-12 months (1.6%), with others reporting a hospital stay of 1-2 weeks (28.3%), 3-4 weeks (22.8%), 1-2 months (25.2%), or 3-4 months (13.4%).

Of the 127 newborns who had additional hospitalizations

TABLE 1 | HI genetic testing results, by HI type.

HI Type	HI Genetic Testing Results		
	Positive for change/ mutation in HI-related gene(s) n (%)	Negative for change/ mutation in HI-related gene(s) n (%)	Total n (%)
Diffuse	53 (61.6)	16 (37.2)	69 (53.5)
Focal	15 (17.4)	2 (4.7)	17 (13.2)
Atypical	5 (5.8)	1 (2.3)	6 (4.7)
Unknown	9 (10.5)	14 (32.6)	23 (17.8)
HI suspected	1 (1.2)	1 (2.3)	2 (1.6)
Undiagnosed form	3 (3.5)	9 (20.9)	12 (9.3)
Total	**86**	**43**	**129**

TABLE 2 | Detected HI genes.

Gene	Participants n (%)
ABCC8 (SUR1)	59 (62.1)
GLUD1 (Glutamate dehydrogenase)	13 (13.7)
KCNJ11 (KIR6.2)	9 (9.5)
GCK (Glucokinase)	5 (5.3)
HNF1A	3 (3.2)
SLC16A1 (MCT1)	2 (2.1)
HNF4A	1 (1.1)
HADH	1 (1.1)
UCP2	1 (1.1)
PMM2	1 (1.1)
Total	**95**

Individuals could choose more than one response.

TABLE 3 | Birth weight and gestational age.

Gestational Age	n (%)	Weight in KG	
		Mean (SD)	[Min, Max]
Less than 28 weeks (extreme prematurity)	1 (.7)	1.25 (N/A)	N/A
28-33 weeks (prematurity)	16 (12.4)	2.46 (.74)	[1.64, 3.88]
34-36 weeks (late prematurity)	22 (17.1)	3.50 (.97)	[1.82, 5.19]
37-42 weeks (term)	90 (69.8)	3.75 (.71)	[1.96, 5.64]
Total participants	**129**		

TABLE 4 | Abnormal blood glucose recorded before leaving facility, by birth weight.

Parent reported results of an abnormal blood glucose test before leaving the birthing facility or before being released to parent care	Birth Weight (kg)			
	Low birthweight (< 2.50)	Average birthweight (2.51 – 4.0)	High birthweight (4.01+)	Total
Yes	8	39	31	78
No	6	23	5	34
Unknown	1	9	3	13
Total	15	71	39	125

TABLE 5 | Age participant's symptoms were first noticed.

Age participant's symptoms were first noticed	Participants n (%)
Unknown	3 (2.1)
Within 1 hour of birth	31 (22.0)
After 1 hour to 24 hours of birth	26 (18.4)
2 days of life	14 (10)
3 – 6 days of life	7 (5.0)
1 – 4 weeks of life	9 (6.4)
5 – 7 weeks of life	2 (1.4)
2 – 6 months old	27 (19.1)
7 – 12 months old	11 (7.8)
1 – 3 years old	7 (5)
4 – 9 years old	2 (1.4)
10 – 17 years old	1 (.7)
18 years +	1 (.7)
Total	**141**

TABLE 6 | Identified signs of hypoglycemia before leaving the birthing facility.

Respondent reported known hypoglycemia risk factors identified prior to or shortly after delivery (before leaving the birthing facility)	Participants n (%)
Unknown	31 (18.3)
No risk factors	48 (28.4)
Family history of a genetic form of a hypoglycemia	8 (4.7)
Hemolytic disease of the newborn (break down of red blood cells)	0 (0)
Polycythemia (high concentration of red blood cells)	4 (2.4)
Abnormal physical features (e.g. midline facial malformations, microphallus)	2 (1.2)
Up to 48 hours of age: Unable to maintain sugar above 50 mg/dL (2,8 mmol/l or 0,5 g/L) before usual feed	80 (47.3)
Over 48 hours of age: Unable to maintain sugar above 60 mg/dL (3,3 mmol/l or 0,6 g/L) before usual feed	56 (33.1)
Total	**169**

Individuals could choose more than one response.

after the initial stay at birth before an HI diagnosis was made, 18.1% (n=23) were hospitalized one other time, 7.1% (n=9) were hospitalized two more times, and 8.7% (n=11) were hospitalized more than three times, including 2.4% (n=3) who were hospitalized more than ten times.

Hospitalizations and Transition to Home

A total of 39.0% (n=55) of respondents reported a change in the participant's medical treatment less than a month after discharge from the hospital after the initial diagnosis, and an additional 26.2% (n=37) reported a change in 1-3 months (**Table 7**). Examples of changes included medication type, dose or schedule, or a feeding regime or diet change. Only 8.5% (n=12) of respondents stated that no change was necessary.

Within the past 12 months of when the respondents answered this question, 40.1% (n=68) of participants required hospitalization due to problems related to blood sugar; 20.6% required four or more stays, and 5.9% were hospitalized seven times or more.

Medication Management

A total of 139 respondents completed the medication management survey and provided information about medication history. Participants have used the following HI medications: diazoxide (82.7%, n=115), octreotide (24.5%, n=34), glucagon (19.4%, n=27), and lanreotide (11.5%, n=16). Of participants whose medical management has included diazoxide, 65.2% (n=75) are currently taking the medication. Of those presently taking diazoxide, 12 respondents reported that participants had a low blood sugar below 70 mg/dL more than once a day, seven reported a low once per day, and five reported a low several times per week.

TABLE 7 | Changes in home management following HI diagnosis and pancreatectomy.

	Change in medical treatment after hospitalization where HI diagnosis was made n (%)	Change in HI management after pancreatectomy n (%)
No change necessary	12 (8.5)	7 (22.6)
Less than 1 month	55 (39.0)	5 (16.1)
1-3 months	37 (26.2)	11 (35.5)
4-6 months	19 (13.5)	4 (12.9)
7-9 months	1 (.7)	0 (0)
10-12 months	2 (1.4)	1 (3.2)
Greater than 1 year	7 (5.0)	1 (3.2)
Unknown	8 (5.7)	2 (6.5)
Total	**141**	**31**

Of the participants who have ever used diazoxide, only 2.7% did not experience any side effects (**Table 8**). The most commonly reported side effects were increased growth of body hair (84.1%), loss of appetite (34.5%), swelling (hands, feet, or both) (25.7%), facial changes (23.9%), and stomach pain or upset (21.2%). A reported 29.2% of respondents for participants on diazoxide reported continued hypoglycemia. Of the 106 respondents who answered, 14.2% indicated that adverse effects caused the participant to stop taking the medication and 12.3% had to stop due to problems with availability.

Surgical Experience

Of 142 participants, 29.6% (n=42) had a pancreatectomy, and another 9.9% (n=14) of families considered a pancreatectomy, but the participant did not have surgery. Of these respondents whose child did not have surgery and reported a known HI type, all (n=11) had diffuse disease. For children who did not have a pancreatectomy, 76.9% of their parents said they preferred medical management, and 23.1% cited health concerns, such as diabetes or complications. Of participants who had a pancreatectomy, 21.4% (n=9) required an additional pancreatectomy.

The total amount of each participant's pancreas removed was 95% or greater for 47.6% (n=20), between 50-94% for 26.2% (n=11), 25-49% for 11.9% (n=5), and less than 25% for the remaining 14.3% (n=6). Of the participants who had 95% or more of their pancreas removed, 25% (n=5) of respondents reported the use of pancreatic enzymes. Everyone over twelve years (n=8) old who had a subtotal pancreatectomy reported diabetes.

TABLE 8 | HI medication side effects.

Side effects	Diazoxide %	Octreotide %	Lanreotide %
None	2.7	30.3	25.0
Loss of appetite	34.5	N/A	N/A
Stomach pain or upset	21.2	24.2	18.8
Changes in sense of taste	8.0	N/A	N/A
Increase in growth of body hair	84.1	N/A	N/A
Headache	7.1	0	6.3
Dizziness	3.5	0	6.3
Skin rash	8.0	N/A	N/A
Facial changes	23.9	N/A	N/A
Swelling (hands, feet, or both)	25.7	N/A	N/A
Racing heartbeat (tachycardia)	15.0	N/A	N/A
Fluid in the lungs	5.3	N/A	N/A
Pulmonary hypertension	6.2	N/A	N/A
Continued hypoglycemia	29.2	42.4	43.8
Hyperglycemia	7.1	24.2	12.5
Nausea	.9	9.1	18.8
Changes in stool	N/A	33.3	43.8
Gallstone/gallbladder sludge	N/A	12.1	18.8
Growth suppression	N/A	6.1	0
Thyroid suppression	N/A	0	0
Necrotizing enterocolitis	N/A	0	0
Injection site problem	N/A	9.1	18.8
Total	**113**	**33**	**16**

Individuals could choose more than one response.

Of 31 participants, 16.1% (n=5) required a change in HI management less than one month after being released to go home after their first pancreatectomy (**Table 7**). An additional 35.5% (n=11) needed a change within 1-3 months. A change included a change in medication type, dose, or schedule, or feeding regime or diet. Only 22.6% (n=7) of respondents reported that no change in HI management was necessary.

Glucose Monitoring

Continuous glucose monitoring systems (CGMs) were used by 45.7% (n=59) of participants, and 76.3% (n=45) of those individuals use CGMs continuously (**Table 9**). For those who responded that a Continuous Glucose Monitor (CGM) was not used, it was because they were not recommended by a health care professional (55.3%, n=42), the device was not covered under medical care benefits (18.4%, n=14), or the device was not affordable (13.2%, n=10).

Of 131 participants, 98.5% did use a glucometer. The two individuals who did not use a glucometer used another device to monitor their blood sugar. A total of 53.5% of participants are either continuously using CGMs or are checking their blood sugar with a glucometer four or more times a day. The most common frequency of glucometer use was 2-3 times a day (34.1%, n=44); however, 11.6% (n=15) of participants check seven or more times a day. Additionally, 34.9% (n=45) of participants who are checking blood sugar with a glucometer continuously wear a CGM.

Twenty respondents reported blood sugars below 70 mg/dL (3.9 mmol/l, 0.7g/L) more than once a day. An additional 23 participants experience lows once per day or several times per week. In addition to lows, 14 participants experience high blood sugar above 180 mg/dL (10 mmol/L, 1.8 g/L) once per week or more frequently. This does not include participants with diabetes or focal HI.

TABLE 9 | Frequency of glucometer and CGM use.

	How often does the participant wear the CGMs			
Average times per day participant uses a glucometer to monitor blood sugar	**Continuously**	**Non-continuously**	**Does not use CGMs**	**Total**
Less than once	5	4	12	21
1 time	7	1	10	18
2-3 times	11	4	29	44
4-6 times	12	3	15	30
7-10 times	8	1	4	13
More than 10 times	1	1	0	2
Unknown	1	0	0	1
Total	**45**	**14**	**70**	**129**

As part of the participant's HI management, 47.2% (n=67) of respondents said they have in-hospital fasting tests. 35.8% (n=24) said that the length of time the participant can fast in-hospital does not correspond to the length of time the participant can fast before blood sugar drops below 70 mg/dL (3.9 mmol/l, 0.7 g/L) at home, whereas 50.7% (n=34) of respondents believed it did correspond to their experience at home, and 13.4% (n=9) were unsure.

During episodes of blood sugar below 70 mg/dL (3.9 mmol/l, 0.7g/L), 29.3% of 123 respondents stated the participant never had symptoms, 33.3% said they rarely have symptoms, 24.5% sometimes have symptoms, 10.6% frequently have symptoms, and only 2.4% always have symptoms. Of the individuals that do experience symptoms of hypoglycemia, the most typical include irritability/mood changes (62.1%, n=54), lethargy/fatigue (51.7%, n=45), clouded thinking/confusion (32.2%, n=28), tremors/shakiness (25.3%, n=22), and headache (25.3%, n=22) (**Table 10**).

Diet and Feeding Management

Of 145 participants, 46.2% (n=67) used nasogastric or orogastric tubes and 32.4% (n=47) used a gastrostomy tube for feeds at some point since HI was diagnosed. Of the 81 participants who have utilized tube feeding to manage blood sugar, 50.6% (n=41) used them continuously. The majority, 69.4% (n=102) of participants, have between four to seven meals or snacks in an average 24-hour period by mouth or tube.

Of 137 respondents, 68.6% (n=94) report feeding issues, including 73.1% of participants with diffuse HI and 75.0% of participants with focal HI. Of the 30 participants with diffuse or focal HI who had a pancreatectomy, 80.0% had feeding issues (**Table 11**). Feeding issues include poor appetite (41.6%, n=57), refusing to eat (40.9%, n=56), reflux (32.1%, n=44), vomiting (27.7%, n=38), and problems with texture (28.0%, n=39).

Of those who reported a feeding issue, 60.6% (n=57) said that the issue has not resolved. For the participants whose feeding issues have resolved, 33.3% (n=12) resolved within the first year of life, 30.6% (n=11) resolved between 1-3 years old, and 13.9%

TABLE 10 | Reported symptoms of hypoglycemia the participant exhibits when blood sugar is low.

What symptoms of hypoglycemia does the participant typically exhibit when blood sugar is low	Participants n (%)
Irritability/Mood Change	54 (62.1)
Anxiety	13 (14.9)
Loud crying	7 (8.0)
Lethargy/Fatigue	45 (51.7)
Headache	22 (25.3)
Clouded thinking/Confusion	28 (32.2)
Poor feeding/appetite	10 (11.5)
Constant hunger	14 (16.1)
Nausea	9 (10.3)
Unusual eye movement/Staring spells	18 (20.7)
Tremors/Shakiness	22 (25.3)
Sweating	21 (24.1)
Seizure	8 (9.2)
Other	9 (10.3)
Total	**87**

Individuals could choose more than one response.

TABLE 11 | Feeding issues regularly experienced by participants.

Has the participant regularly experienced any feeding issues regularly	Participants n (%)
No feeding issues	43 (31)
Feeding issues	94 (67)
Poor appetite	57 (42)
Refusing to eat	56 (41)
Reflux	44 (32)
Problems with texture	39 (28)
Gagging	37 (27)
Vomiting	38 (28)
Uncoordinated oral skills	28 (20)
Slow eating	37 (27)
Coughing	23 (17)
Overeating	12 (9)
Total	**137**

Individuals could choose more than one response.

(n=5) were over the age of seven before the feeding issues resolved. For participants where the feeding issues have not resolved 36.8% (n=21) were between 0 to 1 year old, 36.8% (n=21) were between 2-5 years old, 15.8% (n=9) were between 6-15 years old, and 10.5% (n=6) are over the age of 16.

The reported average number of hours participants can fast without their blood sugar dropping below 70 mg/dL (3.9mmol/l, 0.7g/L) in the daytime and overnight varied considerably among the 123 individuals who responded (**Table 12**).

Development and Neurology

Of 135 respondents who completed the developmental survey, 44.4% (n=60) reported delays in participant reaching developmental milestones. Of all respondents, 25.9% (n=35) reported a delay in talking/speech; other reported delays included walking (14.1%, n=19), feeding (13.3%, n=18), crawling (13.3%, n=18), and fine motor skills (11.9%, n=16) (**Table 13**).

TABLE 12 | Daytime and overnight fasting and low blood sugar.

Average number of hours participant can fast before his or her blood sugar drops below 70 mg/dL (3.9mmol/l, 0.7g/L)	Daytime n (%)	Overnight n (%)
0	4 (3.3)	3 (2.4)
1	4 (3.3)	0 (0)
2	9 (7.3)	4 (3.3)
3	22 (17.9)	7 (5.7)
4	30 (24.4)	7 (5.7)
5	10 (8.1)	4 (3.3)
6	7 (5.7)	8 (6.5)
7	0 (0)	4 (3.3)
8	4 (3.3)	10 (8.1)
9	1 (.8)	4 (3.3)
10	2 (1.6)	10 (8.1)
11	0 (0)	5 (4.1)
12	2 (1.6)	15 (12.2)
More than 12 hours	12 (9.8)	30 (24.4)
Unknown	16 (13.0)	12 (9.8)
Total	**123**	**123**

Of 130 respondents, 37.7% (n=49) reported a chronic neurological problem they feel is due to prolonged hypoglycemia (**Table 13**). The problems include developmental delay (19.2%, n=25), sensory processing challenges (19.2%, n=25), learning disability (10.0%, n=13), poor coordination (8.5%, n=11), and behavior problems (7.7%, n=10).

A total of 31.2% (n=44) of respondents reported a neurological diagnosis, including 12.8% (n=18) of participants who have been diagnosed with epilepsy. Other reported diagnoses include learning disability (9.9%, n=14), anxiety (8.5%, n=12), ADHD (7.8%, n=11), and sensory processing (7.1%, n=10) (**Table 13**).

Patient and Caregiver QOL

Of 23 adult participants, 52.2% (n=12) report they "very often" or "quite often" feel HI rules their lives, while 39.1% (n=9) report they "seldom" or "never" feel HI rules their lives. When asked to describe their experience with the daily management of the HI-related condition, adult participants and caregivers reported it was demanding, complicated, and disruptive (**Table 14**). Parents of children under 5 were more likely than parents of older children to indicate these responses. In contrast, a higher percentage of parents of older children indicated that the management of HI was simple (13% for parents of children under five versus 22.2% for parents of children over 12).

The experience of obtaining a diagnosis was highly variable, with 27.6% (n=37) saying it was "very good," but 22.4% (n=30) said it was "difficult," and 18.7% (n=25) said it was "very difficult."

The financial impact of HI had a varied effect on households; 31.6% (n=42) of respondents said their household income was negatively impacted "quite a lot" or "very much" due to the participant's HI related condition, and only 11.3% (n=15) indicated they were not at all impacted. Of parents who responded, 62.1% (n=82) said that their work/career plans

TABLE 13 | Reported developmental delays and neurological problems.

Reported neurological problems or delays in developmental milestones	Participants n (%)
Delays in reaching developmental milestones	135
Talking/speech	35 (25.9)
Walking	19 (14.1)
Feeding	18 (13.3)
Crawling	18 (13.3)
Fine motor skills	16 (11.9)
Sitting	12 (8.9)
Motor skills	3 (2.2)
Gross motor skills	9 (6.7)
Rolling over	6 (4.4)
Standing	4 (3.0)
Globally delayed	5 (3.7)
Focus/attention	2 (1.5)
Adaptive skills	5 (3.7)
Reading	2 (1.5)
Chronic neurologic problems felt to be due to prolonged hypoglycemia	130
Developmental delay	25 (19.2)
Sensory processing	25 (19.2)
Learning disability	13 (10.0)
Poor coordination	11 (8.5)
Behavior problems	10 (7.7)
Seizure disorder	9 (6.9)
Hypotonia	9 (6.9)
Strabismus	9 (6.9)
Memory processing	9 (6.9)
Low vision	5 (3.8)
Other	3 (2.3)
Cerebral palsy	3 (2.3)
Movement disorder	2 (1.5)
Developmental delay	25 (19.2)
Neurological disorders reported	141
Epilepsy	18 (12.8)
Learning Disability	14 (9.9)
Anxiety	12 (8.5)
ADHD	11 (7.8)
Sensory Processing	10 (7.1)
Mental Health	6 (4.3)
Autism	5 (3.5)
OCD	4 (2.8)
Auditory Processing	4 (2.8)
Migraine	3 (2.1)
Cerebral Palsy	3 (2.1)
Depression	3 (2.1)
Dyslexia	2 (1.4)
Blindness	1 (.7)
Deafness	1 (.7)
Pervasive Disorder	1 (.7)

For each question, individuals could choose more than one response.

changed "somewhat," "quite a lot," or "very much" due to the participant's HI-related condition. Additionally, 19.1% (n=25) of individuals said they missed work or school "quite a lot" or "very frequently" due to their child's HI.

Of 133 parents, 31.6% (n=42) said they "always" worry because of the participant's HI, and an additional 51.9% (n=69) said they worry "very" or "quite often" (**Table 15**). Despite these challenges, 19.4% (n=26) of respondents rated their quality of life as "excellent," while 42.5% (n=57) said it was "very good," 22.4% (n=30) said "good," 13.4% (n=18) said "fair," and only 2.2% (n=3) said it was "poor."

TABLE 14 | Parent/caregiver and patient experience with daily management of HI.

	Parent/caregiver experience with daily management of the participant's HI	Participant experience with the daily management of their HI
Simple	22 (16.5)	5 (21.7)
Manageable	75 (56.4)	13 (56.5)
Demanding	56 (42.1)	7 (30.4)
Complicated	33 (24.8)	7 (30.4)
Disruptive	23 (17.3)	6 (26.1)
Other	2 (1.5)	0 (0)
Total	**133**	**23**

Individuals could choose more than one response.

TABLE 15 | Parent/caregiver experience with worry because of the participants HI, by age groups.

Do you worry because of the participant's HI or HI-related condition	Under 5 years old	5 – 11 years old	12 years +	Total
Always	29	9	4	42
Very Often	11	12	6	29
Quite Often	19	9	12	40
Seldom	7	8	5	20
Never	2	0	0	2
Total	**68**	**38**	**27**	**133**

DISCUSSION

Limitations

Most rare disease registries experience patient recruitment and retention challenges (5, 15), but CHI has made an effort to mitigate this through on-going engagement strategies, including presentations at family conferences, messages on social media, engagement with medical professionals to encourage recruitment, and direct email reminders. HIGR Participants are more likely to be from North America and younger than non-participants in the HI community. There is the potential for recall bias; however, rare disease patients and caregivers are very involved in disease management (17–19), which increases the likelihood of accurate responses.

Individuals who join the registry are likely to be more connected to CHI compared to those not participating, which could result in selection bias. Although there is some evidence generally in rare disease registries that participants have more severe forms of the diseases (13, 15), there is not enough information to evaluate if this is the case in HI. In the future we hope to recruit more individuals who have been cured, including transient patients and those who are post-pancreatectomy, as they are currently under-represented.

CHI is exploring other ways to collect data related to the overall burden of disease and experience of patients, combining these efforts with HIGR recruitment will provide a way to further characterize the burden of disease. Another limitation is that HIGR is currently only available in English. Therefore, the results may not be fully generalizable to all HI patients. However, plans are underway to include additional languages on the platform in the future.

In some cases, we report small numbers of cases, these are seen as individual vignettes of patient experiences that can act as a starting point to better understand the overall experience of living with HI. The HIGR team also has data validation and data quality standards to monitor the data's reliability.

Implications for HI Patients and Research

The early results of the insights generated by the only patient-reported data source are consistent with the literature and other studies completed, including the genetics (1, 2), the reported side-effects of current medications (3, 20, 21), the timing and development of post-pancreatectomy diabetes (22, 23), and the neurological implications associated with prolonged hypoglycemia (2–4).

The number of respondents who reported an unspecified type of HI, even when radiology, medical management, or surgery provided more information about type, indicates that the nomenclature of HI is not universally adopted in the patient community. Focal versus diffuse is important for surgery, especially since focal HI can be cured with surgery, but overall, these types may not resonate with patients because of the heterogeneity of severity in the diffuse group. It is important to understand what patients call their disease and to understand the overall experience across and within subtypes, research that can be furthered through the registry and natural history studies.

Of the newborns in HIGR, 30.2% were born premature. Annually, 11% of babies are born preterm globally (24). Therefore, the rate of preterm babies is much higher in HIGR than worldwide. According to the World Health Organization (WHO), the average weight of a full term baby is 3.2 kg for girls and 3.3 kg for boys, with a range of 2.5-4 kg (25). Babies that weigh less than 2.5 kg are considered low birth weight, regardless of gestational age. In the general population this is bottom 3rd percentile of all babies, but in HIGR 12.4% of babies were under 2.5 kg. Babies over 4 kg are in the top 97th percentile and 30.0% of all HI babies were born over 4 kg.

Additionally, this patient reported research adds many insights to the existing literature and provides new insights for timely diagnosis. Although babies born with HI were within the average weight ranges outlined by WHO, overall, they were on the higher end of the spectrum. In many hospitals, low weight and high weight babies may have their blood sugar screened, however, 50% of normal weight baby participants who were later diagnosed with HI did not have a blood glucose test before leaving the hospital. That normal weight babies in the general population are not typically screened at birth for low blood sugar increases the likelihood of neurologic sequelae and death in those born with HI. Furthermore, 33.9% of respondents reported hospitalization readmission for symptoms of hypoglycemia prior to diagnosis. Adopting the concept of blood sugar as a vital sign will help identify those newborns experiencing hypoglycemia. This is especially critical as 28.4% of newborns with HI in HIGR had no known risk factors of hypoglycemia before leaving the birthing facility.

HIGR also illuminates the on-going fluctuations in care during the transition from hospital to home and points to some of the reasons for these changes. It is revealing that only 19.4% of participants reported having a fasting study before leaving the birthing facility. Even for the individuals who had a fasting study, 66.7% required a change in management within three months. HIGR also shows the limits of in hospital fasting; 35.8% of individuals do not believe their in-hospital fasting experience was reflective of their experience at home.

After hospitalization, including for surgery, many respondents report a change in medical treatment within a few months of discharge, with 51.6% reporting a change within three months post-pancreatectomy and 65.2% reporting a change in medical treatment within three months of discharge from the hospital. This shows a need to prepare HI families for the on-going challenges of managing HI after they are discharged from the hospital and the need for constant vigilance by patients and families to monitor the patient's condition.

The burden of finding a proper diagnosis and a successful disease management regime is reflected in the length of hospitalizations (three weeks or longer for over 62.2% of individuals) and the number of hospitalizations (three or more hospitalizations for 8.7% of all individuals). The burden of hospitalization is an on-going issue for individuals with HI, with 40.1% of respondents reporting at least one hospitalization within the past 12 months directly related to blood sugar.

One implication of the registry data is the need for new innovative treatments, as indicated by the reported side effects of current medications and the continued hypoglycemia among participants. One current categorization of HI in the literature is diazoxide responsive or diazoxide unresponsive; however, respondents report 32% of participants currently taking diazoxide experience low blood sugar below 70 mg/dL (3.9 mmol/l, 0.7g/L) multiple times a week or more frequently. This challenges the concept of diazoxide responsive and underscores the need for new options for medical management. Additionally, of 107 participants, 14.0% (n=15) had to stop taking diazoxide due to adverse effects.

Another insight is that for severe diffuse HI, a pancreatectomy is a choice fraught with significant negative health consequences. For those who have a subtotal pancreatectomy, individuals are trading one disease for another as eventually after their pancreatectomy, they develop diabetes and many also require pancreatic enzymes (22, 23, 26, 27). However, this tradeoff is often the only logical option to save the brain. These conditions may be an immediate change for some patients, while others may have eight years or more before diabetes occurs. This explains why individuals under twelve who underwent a subtotal pancreatectomy may not yet report diabetes.

HI patients are currently using glucose monitoring devices, such as CGMs by 43.4% of participants and glucometers by 98.5% of participants. Access to these devices is a problem, as is trust in CGMs by medical providers for some participants. Monitoring blood sugars is critically important, especially since only 13% of participants sometimes or frequently have symptoms when experiencing hypoglycemia. This underscores the overall reliance on blood sugar monitoring and the need to have accurate approved devices and tools to support patients and families.

Many individuals within the HI community have ongoing feeding issues, some requiring tube feeding regimes to stabilize the participant's blood sugars either continuously (28.3% of all individuals) or overnight (26.9%). For 60% of participants, these feeding issues have not resolved. The feeding frequency is also an added part of the patient and family burden. In addition to those who required continuous feeds, many respondents report needing to feed every few hours to avoid drops in blood sugar, however, a constant challenge is refusal to eat or other feeding challenges. This represents a burden on the family as planning for meals needs to be a constant consideration.

The most-reported neurological outcomes of HI include 37.7% delays in developmental milestones and 31.2% receiving a specific neurological diagnosis. Swift action by medical professionals is key to reduce prolonged hypoglycemia, and increased awareness, and clinical guidance are crucial to prevent these and other issues.

On a positive note, parents describe a high quality of life despite these challenges. Parents are able to distinguish between burden of disease and happiness. Parental descriptions of ongoing worry, the demands of the disease, and financial impacts provide more insight into the burden of disease that accompanies the overall fears of hypoglycemia and the burden balancing complex medical and feeding regimes. For those whose children were diagnosed within the first few days of life, living with a child diagnosed with a rare disease is all the child and their family know, and rare disease families are known for their resilience.

Future Directions

In addition to the potential of the CRN to provide infrastructure to facilitate partnerships with researchers at different institutions and companies to explore research questions that can be explored with the registry in the future, some specific projects are already underway. Any academic researcher can request data from HIGR to support their research.

Two pilot projects are supported through the Million Dollar Bike Ride (MDBR), a project of the Orphan Disease Center at the University of Pennsylvania. The 2020 pilot grant is funding MaxHIGR, a partnership between researchers at Northern Congenital Hyperinsulinism Service (Manchester, England), Collaborative Alliance on Congenital Hyperinsulinism (Magdeburg, Germany), Great Ormond Street Hospital for Children (London, England), The Children's Hospital of Philadelphia (Pennsylvania, USA), Cook Children's Hospital (Texas, USA), University Medical Center - National Research Center for Maternal and Child Health (Nur-Sultan, Kazakhstan), other academic researchers worldwide, and CHI to include physician-reported data elements in HIGR. The project is being conducted as a pilot to expand the data generated through HIGR and increase the universe of information available to researchers and regulatory bodies in the future. MaxHIGR will allow for the possibility of validating the patient-reported dataset, thus enhancing the value, credibility, and impact of HIGR. The secondary aims of MaxHIGR are to validate the parent QOL questionnaires clinically and build a prototype model for an integrated database, combining fields from patient-populated and clinician-provided information.

The 2021 MDBR recipients from the Children's Hospital of Philadelphia are also utilizing HIGR as part of a multi-center observational study to investigate the natural history of hyperinsulinism hyperammonemia syndrome. The goal is to increase understanding of disease and outcomes for patients.

To further support needed HI research, the HIGR investigators are expanding the scope of data collection to include CGM data. Blood sugar levels are of critical importance to HI patients. Understanding patterns alongside other patient-reported outcomes from a wide range of patients is valuable to clinicians, drug developers, and families. Many patients use CGMs to monitor their blood sugar and rely on the insights provided to manage their disease and prevent lows. Currently, CGMs are not approved for use in HI. Generating additional evidence related to their use and impact on disease management could help lead to device designation and increased access for HI families.

A final additional avenue for HIGR is to aid in the drug development process more directly by collecting post-marketing information and as a control arm in clinical trials. The registry can create substudies to follow a subset of patients, such as individuals on a specific treatment, and to collect existing or specifically developed surveys. Looking at sub-populations and their experience across time can provide a naturally occurring control group for a clinical trial. Controls are essential for scientific understanding, but there are ethical implications for not allowing individuals to try potential new treatments in a rare disease clinical research study. In HI, it is very difficult to conduct blinded studies as without the standard of care therapy individuals would likely experience hypoglycemia from the very beginning of the study and throughout the duration. Additionally, as previously discussed, a participant's medical management and needs change frequently, which makes it difficult to even use prior historical data from the individuals' own experience as a control. A larger pool of historical data or active controls from individuals who cannot logistically join an active study can help provide the needed evidence. The FDA has provided draft guidance to industry to utilize registries in this way (11), but patient organization registries are uniquely positioned to support trials and should receive similar guidance and support to further clinical development within their disease state.

CONCLUSION

HIGR has already shown the potential to capture and disseminate information that can generate new insights into HI, including patient outcomes and caregiver QOL. The data presented above underscores the need for new treatments for individuals that have fewer side effects and reduce hypoglycemia events. CHI also advocates for the adoption of blood sugar as a vital sign, to further identify and diagnose patients and prevent brain damage. This information can help drive new research for

treatments and cures and support clinical trials. Understanding the natural history of the disease can also guide standards of care. The data generated through HIGR provides an opportunity to improve the lives of all those affected by HI.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by North Star Review Board. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

TP determined which data elements to include in the manuscript, directed the data analysis process, and wrote the initial draft. She was involved in all revisions to the text. JR, together with the other founding investigators, conceptualized and designed the questionnaires in HIGR. JR was the senior author on this paper leading the interpretation and contextualization of key findings in the paper and conducted major revisions and edits to the text. MM generated data reports, analysis, and tables for the manuscript and provided text for the future directions section of the paper. JS provided comments and critical revisions of the manuscript. All authors read and approved the final manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Stanley CA. Perspective on the Genetics and Diagnosis of Congenital Hyperinsulinism Disorders. *J Clin Endocrinol Metab* (2016) 101(3):815–26. doi: 10.1210/jc.2015-3651
2. Rosenfeld E, Ganguly A, De Leon DD. Congenital Hyperinsulinism Disorders: Genetic and Clinical Characteristics. *Am J Med Genet C Semin Med Genet* (2019) 181(4):682–92. doi: 10.1002/ajmg.c.31737
3. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ. Therapies and Outcomes of Congenital Hyperinsulinism-Induced Hypoglycaemia. *Diabetes Med J Br Diabetes Assoc* (2019) 36(1):9–21. doi: 10.1111/dme.13823
4. Arnoux J-B, Verkarre V, Saint-Martin C, Montravers F, Brassier A, Valayannopoulos V, et al. Congenital Hyperinsulinism: Current Trends in Diagnosis and Therapy. *Orphanet J Rare Dis* (2011) 6:63. doi: 10.1186/1750-1172-6-63
5. Boulanger V, Schlemmer M, Rossov S, Seebald A, Gavin P. Establishing Patient Registries for Rare Diseases: Rationale and Challenges. *Pharm Med* (2020) 34(3):185–90. doi: 10.1007/s40290-020-00332-1
6. RE Gliklich, MB Leavy, NA Dreyer eds. *Registries for Evaluating Patient Outcomes: A User's Guide. 4th ed.* Rockville (MD: Agency for Healthcare Research and Quality (US (2020). Available at: http://www.ncbi.nlm.nih.gov/books/NBK562575/.
7. Richesson R, Vehik K. Patient Registries: Utility, Validity and Inference. *Adv Exp Med Biol* (2010) 686:87–104. doi: 10.1007/978-90-481-9485-8_6
8. Jansen-van der Weide MC, Gaasterland CMW, Roes KCB, Pontes C, Vives R, Sancho A, et al. Rare Disease Registries: Potential Applications Towards Impact on Development of New Drug Treatments. *Orphanet J Rare Dis* (2018) 13:154. doi: 10.1186/s13023-018-0836-0
9. Basch E, Bennett AV. Patient-Reported Outcomes in Clinical Trials of Rare Diseases. *J Gen Intern Med* (2014) 29 Suppl 3:S801–803. doi: 10.1007/s11606-014-2892-z
10. Bellgard MI, Snelling T, McGree JM. RD-RAP: Beyond Rare Disease Patient Registries, Devising a Comprehensive Data and Analytic Framework. *Orphanet J Rare Dis* (2019) 14(1):176. doi: 10.1186/s13023-019-1139-9
11. *Real-World Data: Assessing Registries to Support Regulatory Decision-Making for Drug and Biological Products Guidance for Industry. FDA-2021-D-1146. [Internet]*. Rockville, MD:U.S. Food and Drug Administration (2021). Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/real-world-data-assessing-registries-support-regulatory-decision-making-drug-and-biological-products.
12. Wu J, Wang C, Toh S, Pisa FE, Bauer L. Use of Real-World Evidence in Regulatory Decisions for Rare Diseases in the United States-Current Status and Future Directions. *Pharmacoepidemiol Drug Saf* (2020) 29(10):1213–8. doi: 10.1002/pds.4962
13. Ali SR, Bryce J, Kodra Y, Taruscio D, Persani L, Ahmed SF. The Quality Evaluation of Rare Disease Registries—An Assessment of the Essential Features of a Disease Registry. *Int J Environ Res Public Health* (2021) 18 (22):11968. doi: 10.3390/ijerph182211968

14. Kodra Y, Weinbach J, Posada-de-la-Paz M, Coi A, Lemonnier SL, van Enckevort D, et al. Recommendations for Improving the Quality of Rare Disease Registries. *Int J Environ Res Public Health* (2018) 15(8):E1644. doi: 10.3390/ijerph15081644
15. Kodra Y, Posada de la Paz M, Coi A, Santoro M, Bianchi F, Ahmed F, et al. Data Quality in Rare Diseases Registries. *Adv Exp Med Biol* (2017) 1031:149–64. doi: 10.1007/978-3-319-67144-4_8
16. RDCA-DAP. Critical Path Institute. Available at: https://c-path.org/programs/rdca-dap/.
17. Currie G, Szabo J. "It is Like a Jungle Gym, and Everything is Under Construction": The Parent's Perspective of Caring for a Child With a Rare Disease. *Child Care Health Dev* (2019) 45(1):96–103. doi: 10.1111/cch.12628
18. Baumbusch J, Mayer S, Sloan-Yip I. Alone in a Crowd? Parents of Children With Rare Diseases' Experiences of Navigating the Healthcare System. *J Genet Couns* (2018) 28(1):80–90. doi: 10.1007/s10897-018-0294-9
19. Pasquini TLS, Goff SL, Whitehill JM. Navigating the U.S. Health Insurance Landscape for Children With Rare Diseases: A Qualitative Study of Parents' Experiences. *Orphanet J Rare Dis* (2021) 16(1):313. doi: 10.1186/s13023-021-01943-w
20. Brar PC, Heksch R, Cossen K, De Leon DD, Kamboj MK, Marks SD, et al. Management and Appropriate Use of Diazoxide in Infants and Children With Hyperinsulinism. *J Clin Endocrinol Metab* (2020) 105(12):dgaa543. doi: 10.1210/clinem/dgaa543
21. Thornton P, Truong L, Reynolds C, Hamby T, Nedrelow J. Rate of Serious Adverse Events Associated With Diazoxide Treatment of Patients With Hyperinsulinism. *Horm Res Paediatr* (2019) 91(1):25–32. doi: 10.1159/000497458
22. Welters A, Meissner T, Grulich-Henn J, Frohlich-Reiterer E, Warncke K, Mohnike K, et al. Characterization of Diabetes Following Pancreatic Surgery in Patients With Congenital Hyperinsulinism. *Orphanet J Rare Dis* (2018) 13:230. doi: 10.1186/s13023-018-0970-8
23. Lord K, Radcliffe J, Gallagher PR, Adzick NS, Stanley CA, De León DD. High Risk of Diabetes and Neurobehavioral Deficits in Individuals With Surgically Treated Hyperinsulinism. *J Clin Endocrinol Metab* (2015) 100(11):4133–9. doi: 10.1210/jc.2015-2539
24. Walani SR. Global Burden of Preterm Birth. *Int J Gynecol Obstet* (2020) 150 (1):31–3. doi: 10.1002/ijgo.13195
25. *Weight-For-Age*. Available at: https://www.who.int/tools/child-growth-standards/standards/weight-for-age.
26. Adzick NS, De Leon DD, States LJ, Lord K, Bhatti TR, Becker SA, et al. Surgical Treatment of Congenital Hyperinsulinism: Results From 500 Pancreatectomies in Neonates and Children. *J Pediatr Surg* (2019) 54(1):27–32. doi: 10.1016/j.jpedsurg.2018.10.030
27. Arya VB, Senniappan S, Demirbilek H, Alam S, Flanagan SE, Ellard S, et al. Pancreatic Endocrine and Exocrine Function in Children Following Near-Total Pancreatectomy for Diffuse Congenital Hyperinsulinism. *PloS One* (2014) 9(5):e98054. doi: 10.1371/journal.pone.0098054

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REVIEW
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Dynamic Methods for Childhood Hypoglycemia Phenotyping: A Narrative Review

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Hypoglycemia results from an imbalance between glucose entering the blood compartment and glucose demand, caused by a defect in the mechanisms regulating postprandial glucose homeostasis. Hypoglycemia represents one of the most common metabolic emergencies in childhood, potentially leading to serious neurologic sequelae, including death. Therefore, appropriate investigation of its specific etiology is paramount to provide adequate diagnosis, specific therapy and prevent its recurrence. In the absence of critical samples for biochemical studies, etiological assessment of children with hypoglycemia may include dynamic methods, such as *in vivo* functional tests, and continuous glucose monitoring. By providing detailed information on actual glucose fluxes *in vivo*, proof-of-concept studies have illustrated the potential (clinical) application of dynamic stable isotope techniques to define biochemical and clinical phenotypes of inherited metabolic diseases associated with hypoglycemia. According to the textbooks, individuals with glycogen storage disease type I (GSD I) display the most severe hypoglycemia/fasting intolerance. In this review, three dynamic methods are discussed which may be considered during both diagnostic work-up and monitoring of children with hypoglycemia: 1) functional *in vivo* tests; 2) *in vivo* metabolic profiling by continuous glucose monitoring (CGM); 3) stable isotope techniques. Future applications and benefits of dynamic methods in children with hypoglycemia are also discussed.

Keywords: hypoglycemia, children, hepatic glycogen storage diseases, functional tests, fasting challenge, continuous glucose monitoring, stable isotopes

INTRODUCTION

Appropriate investigation of the etiology and simultaneous management in children with hypoglycemia is paramount to prevent (irreversible) brain injury or even death (1), although controversy remains on the definition (e.g., diagnostic plasma glucose threshold, definition of at–risk neonates) of childhood hypoglycemia (2–4). From a pathophysiological perspective,

hypoglycemia is caused by an imbalance between the amount of glucose molecules entering the blood compartment and the glucose clearance. A continuous supply of glucose is crucial for the function and development of the brain, because of the absence of sufficient cerebral energy stores in the form of glycogen, protein or fat. Glucose oxidation is responsible for ~ 70% of the energy production in the brain, whereas lactate and ketone bodies can function as important alternative fuels. Hypoglycemia causes energy deficiency in brain cells ultimately leading to brain cell death due to secondary molecular mechanisms, such as activation of neuronal glutamate receptors, oxidative stress, neuronal zinc release and activation of poly-ADP-ribose polymerase-1 (5, 6). Clinically, untreated hypoglycemia can lead to serious neurologic morbidity (encephalopathy, convulsions, coma, developmental delay), and even mortality (7). In individuals with a possible, underlying disorder, unrecognized hypoglycemia may accelerate and aggravate the metabolic decompensation resulting in poor outcomes.

Multiple metabolic pathways cooperate to maintain normal blood glucose concentrations in the fed and fasted state, such as glycogenolysis, gluconeogenesis, mitochondrial fatty acid oxidation, ketogenesis and ketolysis, ensuring energy homeostasis. These pathways are tightly regulated by hormonal (e.g., insulin, glucagon, cortisol, and growth hormone) and autonomic (e.g., catecholamines) responses (8) to maintain normoglycemia. Besides acquired conditions (e.g., insufficient supply/increased loss of nutrients, dysmaturity, drugs, hypothermia, sepsis), hypoglycemia may be explained by a genetic defect of these mechanisms that normally guarantee glucose homeostasis during feeding and fasting. Hypoglycemia is an important feature of several inherited metabolic disorders (IMDs) characterized by a history of childhood fasting intolerance (9).

Compared to adults, children are at an increased risk of developing hypoglycemia, as they have smaller hepatic glycogen and muscle protein stores, together with an increased energy expenditure and higher brain/body weight ratio (10). Consequently, most young children are not able to maintain normoglycemia during more than 24 hours of fasting (11–16). Studies with stable isotope techniques have quantified endogenous glucose production (EGP) in newborns (17) and older children (18). Huidekoper et al. designed a non-linear regression model for EGP from infancy to adulthood (19). **Table 1** illustrates that glucose homeostasis is tightly controlled in an age-dependent manner, with a small glucose pool in the blood compartment (row D) and high glucose turnover rate (row E).

TABLE 1 | Relationship between age, blood glucose pool and endogenous glucose production (EGP).

A	Age (years)	1	3	5	10	15
B	Body weight (kg)[a]	13	18	23	35	48
C	Blood volume (ml)[b]	1,000	1,400	1,800	2,800	3,800
D	Blood pool of glucose (mg)	721	1,009	1,297	2,018	2,738
E	EGP *(mg/kg/min)*[c]	8.2[d]	6.1	5.1	3.5	2.7
	number of sugar cubes per day[d]	34	35	37	40	41

[a], based on 'the APLS formula' BW = [Age(years) + 4] x 2.5;
[b], based on 'the APLS formula' BV = 80 ml/kg x BW(kg);
[c], based on (19);
[d], based on (20).

The most used diagnostic approach to childhood hypoglycemia consists of pattern recognition by combining a variety of information, including medical history (e.g., age at onset, relation with food), physical examination (e.g., growth charts, liver size), and laboratory tests. If critical samples (i.e. samples collected during metabolic stress) of plasma and urine can be obtained, static or single-point concentrations of biomarkers (e.g., blood glucose, lactate, gases, free fatty acids, ketones, acylcarnitines, amino acids, insulin, cortisol, growth hormone, and urine organic acids) can provide crucial information to establish an etiological diagnosis (21). When critical samples are not available or inconclusive, next-generation sequencing may further direct the diagnostic process. Several diagnostic algorithms exist for the clinical problem of childhood hypoglycemia (1, 3), but these schemes are often not sufficiently specific. Moreover, they underestimate the complexity of the swiftly changing clinical signs and concentrations of different fuels in the blood compartment. Hence, many hypoglycemic children do not fit these textbook schemes, and additional investigation is often required. In the end, an important group of individuals is diagnosed with idiopathic ketotic hypoglycemia (22, 23).

In this narrative review, we illustrate several dynamic methods which may be considered in the diagnostic work-up and monitoring of children with hypoglycemia. Three methods are presented: 1) functional *in vivo* tests; 2) *in vivo* metabolic profiling by continuous glucose monitoring (CGM); 3) stable isotope tracing, focusing on glycogen storage disease type I (GSD I). Future applications and benefits of dynamic methods in children with hypoglycemia are also discussed.

FUNCTIONAL *IN VIVO* TESTS

For decades, functional *in vivo* tests have been used for the etiological clarification of fasting intolerance, unexplained hypoglycemia, and to diagnose specific IMDs (**Table 2**). In principle, these tests were particularly relevant as biochemical abnormalities might only appear intermittently in many IMDs. In times when diagnostic enzymatic studies and DNA studies were not routinely available, assessment of blood lactate curves after hexose (e.g. glucose, fructose and galactose) loading was used as a screening procedure in patients suspected of hepatic GSDs (24–26). The results of these tests combined with the assessment of response of blood glucose to glucagon injection provided major clues to identify the hepatic GSD subtype. As an example, the combination of increased blood lactate levels observed after oral glucose administration together with no response of blood glucose to intramuscular glucagon injection pointed to GSD III. Subsequently, the abnormally increasing lactate curves after ingestion of both fructose and galactose in GSD I patients has become one of the pillars, on which the current dietary recommendations are based. If performed, functional *in vivo* tests are conducted strictly adhering to a

TABLE 2 | Main *in vivo* functional tests used in children with hypoglycemia. Historical indications are shown.

Functional *in vivo* test	Historical indications
Explorative controlled fasting test	Assessment of fasting tolerance in situations of childhood hypoglycemia or fasting intolerance, before performing an extended controlled fasting test
Extended controlled fasting test	Clarification of hypoglycemia in FAOD, disorders of ketogenesis/ketolysis and some endocrinopathies
Intravenous glucagon test	Differentiation of hypoglycemia
Oral glucose loading test	Hypoglycemia or moderate/intermittent hyperlactatemia of unknown origin (e.g., hepatic GSDs, disorders of gluconeogenesis, PDH deficiency, hyperinsulinemia, mitochondrial disorders, SGLT1 deficiency)
Oral galactose loading test	SGLT1 deficiency Hepatic GSDs (GSD I excepted)
Oral fructose loading test	Hereditary fructose intolerance SGLT1 deficiency Differentiation of the different forms of PDH deficiency
Intravenous fructose loading test	Hereditary fructose intolerance Fructose-1,6-bisphosphatase deficiency
Oral protein/leucine tolerance test	Hyperinsulinism
Fat loading test	FAOD
Phenylpropionate loading test	Medium-chain acyl-coenzyme A dehydrogenase deficiency

FAOD, fatty acid oxidation disorders; GSDs, glycogen storage diseases; PDH, pyruvate dehydrogenase complex.

defined protocol to maximize the reliability and minimize the risks for patients. Functional *in vivo* tests should only be performed in experienced centers and under close medical supervision. Protocols for several functional *in vivo* (provocation and/or loading) tests can be found elsewhere (9, 27).

In theory, evaluation of metabolic changes in response to an explorative or extended controlled fasting test may be helpful to reach the etiological diagnosis in children with hypoglycemia. Fasting induces several hormonal and metabolic responses to ensure an endogenous supply of energy after cessation of exogenous intake (28). Due to the complexity of the (patho) physiological response to fasting, complete hormonal and metabolic investigations should be performed before and at the end of the monitored fasting. Spare serum/urine samples should also be stored for any additional tests to be performed afterwards. Bedside monitoring of these laboratory parameters, and in particular during the extended controlled fasting test, vital parameter monitoring and CGM should also be performed. Under these circumstances, the maximal duration of the fasting is based on the clinical history (expected fasting tolerance) and on the child's age (usually 12-16 hours at 6-12 months, 18 hours at 1-2 years, 20 hours at 2-7 years, 24 hours in children > 7 years). The fasting should be stopped at any time if the glucose concentration is below 2.6 mmol/L or the patient develops symptoms of hypoglycemia (21).

Even before the era of expanded newborn screening and next generation sequencing, the diagnostic yield of fasting studies has been limited. Morris et al. confirmed an endocrine disorder (n=15) or an IMD (n=15) in a group of 138 patients undergoing fasting studies (29). Bappal and Mula-Abed diagnosed an endocrine disorder (n=21) or an IMDs (n=31) in 96 patients subjected to diagnostic fasting tests (30). Both studies did not provide detailed information on confirmatory diagnostic testing in their patients. More recently Graves et al. showed that 4/91 fasting challenges performed in children with suspected or documented hypoglycemia resulted in an etiological diagnosis (i.e., hepatic GSD, partial ACTH deficiency, panhypopituitarism, isolated central hypothyroidism) (31).

Although *in vivo* functional tests have the advantage of triggering metabolic stress in a supervised setting, they are burdensome and inconvenient for young patients and their parents. If IMDs are not yet excluded, these tests can cause serious complications in unexperienced hands (e.g., worsening of hypoglycemia, accumulation of toxic metabolites). Therefore, psychological burden due to unexpected hypoglycemia for patients and their families should be balanced against the stress and risks associated with *in vivo* functional testing. Additionally, *in vivo* functional tests pose a significant organizational and logistical burden (e.g., need for a fully equipped laboratory and a dedicated medical team) by including several samples to be collected at specific time points (before and after the challenge), under strictly supervised conditions, and requiring hospital admission.

Based on these considerations and because indications have become more restricted, most of the *in vivo* functional tests have fallen into disuse. A few tests are nowadays even abandoned and considered obsolete (9). Historical indications for functional *in vivo* tests have been shifted with the increased awareness for rare diseases, the development of diagnostic algorithms and refined basal metabolic investigations (such as organic acid analysis, plasma acylcarnitines, specific enzyme analysis, *in vitro* metabolic flux analysis in cultured skin fibroblasts), introduction of expanded population newborn screening, and implementation of next-generation sequencing. Still, some functional *in vivo* tests -with slight modifications - may be useful to provide phenotypic information to support genetic variant classification, or to define outcome in clinical trials. In such situations, these tests remain the major (monitoring) methods allowing the assessment of metabolic changes *in vivo* in response to external factors (e.g., metabolic stress, treatment). In addition, controlled fasting test may guide the diagnostic process in suspected congenital hyperinsulinism when critical samples are not available (e.g., molecular genetic testing, ^{18}F-DOPA positron emission tomography) (32, 33).

IN VIVO METABOLIC PROFILING BY CONTINUOUS GLUCOSE MONITORING

Continuous glucose monitoring (CGM) constitutes another dynamic method in the diagnostic work-up and monitoring of children with hypoglycemia. Progress has been made since CGM was firstly described in 1979 for a patient with insulinoma (34). Modern CGM devices consist of a subcutaneous sensor and a

TABLE 3 | Previous studies using continuous glucose monitoring (CGM) in hepatic GSD patients.

Reference	Device	Country	Population (n. of patients)	Age (range in years)	GSD subtype
Hershkovitz ea. J Inherit Metab Dis. 2001 (42).	MiniMed (Medtronic)	Israel	4	2-15	Ia
Maran ea. Diabetes Metab Res Rev. 2004 (43).	Glucoday® (Menarini)	Italy	4	14-47	Ia
			1	22	Ib
			1	10	III
White ea. J Inherit Metab Dis. 2011 (44).	iPro™ (Medtronic)	UK	1	6	0
			6	0-13	Ia
			2	0-3	Ib
			7	4-20	III
			4	5-16	IX
			2	2-24	XI
Kasapkara ea. Eur J Clin Nutr. 2014 (45).	MiniMed (Medtronic)	Turkey	15	2-18	Ia
			1		Ib
Herbert ea. J Inherit Metab Dis. 2018 (46).	Dexcom G4 Platinum (Dexcom)	USA	7	2-56	Ia
			2	9-17	Ib
			6	6-44	III
			5	7-17	IX
Kaiser ea. Mol Gen Metab. 2019 (47).	iPRO2® (Medtronic) Guardian® (Medtronic) FreeStyle Libre® (Abbott)	Switzerland	12	11-49	Ia
			2		Ib
Peeks ea. J Inherit Metab Dis. 2021 (48).	Dexcom G6 (Dexcom)	Netherlands	1	9	Ia
	Dexcom G4 (Dexcom)		12	2-22	Ia, III, IX
	Dexcom G6 (Dexcom)		3	2-11	Ib

receiver. A wearable sensor is attached to the skin and measures glucose (with variable frequency) through a subcutaneous needle inserted in the interstitial fluid. *Via* an enzymatic technology (glucose oxidase), the sensor generates an electric current proportional to the glucose concentration in the interstitium. The electric current flows through a continuously shifting algorithm to generate a glucose value which is eventually transmitted to the receiver (receiving unit or compatible smartphone). The algorithm is finetuned *via* calibration by patient or factory to ensure accuracy over the recording periods (35). Major advantages of CGM are the availability of real-time data, continuous data (density), alarming function, at home use, and data-sharing with caregivers and health care providers, and it being a relatively non-invasive technique (no or fewer finger sticks).

Currently, CGM devices are only licensed for patients with diabetes mellitus (DM), for whom CGM-related outcome parameters (e.g., time-in-range and time-above-range) are defined to guide dietary changes and/or medication adjustments (36). Additionally, CGM has been used in clinical research to assess glucose metabolism in various conditions, including acute coronary syndrome (37) and individuals following an intermittent fasting dietary regimen (38). Evidence on its benefit is also accumulating in endocrine disorders (39, 40) and IMDs associated with hypoglycemia (41), particularly in hepatic GSDs (**Table 3**).

A significant correlation between CGM values and capillary glucose values has been found in patients with GSD I (42, 46). CGM can unveil both unrecognized (nocturnal) hypoglycemia and hyperglycemia and hence, provide information on the effect of the dietary regimen on glucose homeostasis in patients with hepatic GSDs (43). By providing visual glucose trends, CGM also has the advantage of generating dynamic, daily data compared to traditional single-point data. Thus, it can improve the home site monitoring (constituting an easier tool for patients compared to traditional methods) (44, 47) and the day-to-day healthcare for patients with hepatic GSDs (45, 46). CGM has also proven a reliable tool to assess the effect of novel/optimized dietary and/or medical treatments in patients with hepatic GSDs (48, 49). Additionally, it can assist the physicians in the assessment of children with hypoglycemia in combination with relevant information on personal and dietary history and appropriate biochemical investigations.

For patients with ketotic fasting intolerance (e.g., ketotic GSDs, idiopathic ketotic hypoglycemia, or any situation in which euglycemic ketonemia may occur, such as patients on ketogenic diets), parallel documentation of point-of-care capillary beta-hydroxybutyrate (BHB) concentrations by a portable ketone meter may provide important additional information. To the best of our knowledge, reference values for capillary BHB during the day are not available in the pediatric population, but information on BHB normal values after overnight fasting can be extrapolated from historical publications (**Table 4**). Most studies provide BHB concentrations after prolonged fasting, but recently, Parmar et al. demonstrated that serum point-of-care (POCT) BHB measurement using a precision Xtra meter are comparable to serum BHB concentrations in healthy children after an overnight fast (51). In 94 children (age range 6 months to 18.7 years) scheduled to undergo elective surgery, POCT BHB concentrations ranged from 0 to 1.1 mmol/l, but unfortunately, detailed information on the correlation between age and BHB concentrations was not provided in their study.

Currently, only the BHB meters can be used for minimally invasive home monitoring to guide dietary management after a working diagnosis has been established. To our opinion, the combination of real-time CGM with alarm function and a

TABLE 4 | Previous studies assessing ketones and glucose concentrations after prolonged fasting in healthy children.

Reference	Study population (n)	Age (years)	Duration of fasting (hours)	Descriptive statistics	Glucose concentrations (mmol/L)	Ketone body concentrations[1] (mmol/L)
Chaussain ea. J Pediatr. 1977 (11).	28	2 - 17	24	- Range	1.7 - 4.3 Blood sugar significantly correlated to age	Ketonuria ranging from 0 to +++ The extent of ketonuria seemed to decrease with age
Wolfsdorf ea. Eur. J. Pediatr. 1982 (50)	23	1.9 - 16.7	24	- Mean and SD - 95% C.I. for BHB at various ages	3.3 ± 0.7	BHB: 2.8 ± 1.3; ACA: 0.4 ± 0.1 95% C.I. BHB.: - 3 years: 2.5-5.5 - 6 years: 2.0-5.0 - 9 years: 1.0-4.0 -12 years: 0.3-3.5 Negative correlation between plasma ketone bodies and age.
Haymond ea. Metabolism. 1982 (12)	15	6.1 ± 0.8	30	- Mean and SD	2.9 ± 0.2	BHB: 3.7 ± 0.4; ACA: 1.3 ± 0.1 Negative correlation between plasma ketone bodies and blood glucose concentrations.
Lamers ea. Clin Chim Acta.1985a (13)	72	3 - 15	14 (overnight)	- Mean and SD - p2.5-p97.5	4.34 ± 0.34 3.72-5.05	BHB: 0.22 ± 0.23; ACA: 0.10 ± 0.05 BHB: 0.07-1.58; ACA: 0.06-0.30 Negative correlation between plasma ketone bodies and age.
Lamers ea. Clin Chim Acta.1985b (14)	13	3 - 5	24	- Median	3.5	BHB: 2.07; ACA: 0.55
	58	6-15	40	- Mean and SD - p2.5-p97.5	3.44 ± 0.44 2.64-4.41	BHB: - 6-11 years: 3.15 ± 1.38; p2.5: 1.36; p97.5: 8.90 - 12 years: p2.5: 1.01; p97.5: 6.58 - 15 years: p2.5: 0.49; p97.5: 3.18 ACA: - 6-11 years: 0.66 ± 0.25; p2.5: 0.33; p97.5: 1.52 - 12 years: p2.5:0.28; p97.5:1.27 - 15 years: p2.5: 0.18; p97.5: 1.81 Negative correlation between plasma ketone bodies and age.
Bonnefont ea. Eur. J. Pediatr.1990 (15)	12	< 1	15	- p10- p90	3.9 - 5.3	BHB: 0.1 - 1.0
			20		3.5 - 4.6	BHB: 0.5 - 2.3
			24		2.7 - 4.5	BHB: 1.1 - 2.8
	27	1 - 7	15		3.5 - 4.8	BHB: <0.1 - 0.9
			20		2.8 - 4.3	BHB: 0.8 - 2.6
			24		2.8 - 3.8	BHB: 1.7 - 3.2
	9	7 - 15	15		4.4 - 4.9	BHB: <0.1 - 0.3
			20		3.8 - 4.9	BHB: <0.1 - 0.8
			24		3.0 - 4.3	BHB: 0.5 - 1.3
van Veen ea. Pediatrics. 2011 (16)	49	0 - 2	15	- Median - p10-p90	4.1 3.1 - 4.8	BHB: 1.00 (0.22-2.34) ACA: 0.42 (0.10-0.87)
			20		3.3 2.8 - 3.9	BHB: 2.23 (0.91-3.31) ACA: 0.42 (0.38-1.05)
	79	2 - 7	15		4.6 3.8 - 5.3	BHB: 0.30 (0.03-1.26) ACA: 0.16 (<0.05-0.43)
			20		4.0 3.0 - 4.8	BHB: 1.19 (0.36-2.56) ACA: 0.46 (0.13-0.91)
			24		3.8 3.0 - 4.8	BHB: 2.01 (0.81-3.54) ACA: 0.71 (0.27-1.14)

(Continued)

TABLE 4 | Continued

Reference	Study population (n)	Age (years)	Duration of fasting (hours)	Descriptive statistics	Glucose concentrations (mmol/L)	Ketone body concentrations[1] (mmol/L)
	39	7 - 18	15		4.9 4.1 - 5.3	BHB: 0.19 ACA: 0.12 <0.02-1.27 <0.05-0.46
			20		4.2 3.3 - 5.2	BHB: 0.62 ACA: 0.29 0.09-2.18 0.09-0.81
			24		4.1 3.5 - 4.9	BHB: 1.31 ACA: 0.50 0.32-2.46 0.22-0.96
Parmar ea. JIMD Rep. 2021 (51)	94	0.5-18.7	Overnight	Range	3.9-6.7	BHB: 0.0 - 1.2

[1]*Assessed in plasma or serum if not otherwise stated.*

[2]*For each age and fasting duration group median values (top line) and p10-p90 range (bottom line) for BHB (left subcolumn) and ACA (right subcolumn) are shown.*

ACA, acetoacetate; BHB, beta-hydroxybutyrate; p2.5, 2.5th percentile; p10, 10th percentile; p90, 90th percentile; p97.5, 97.5th percentile; SD, standard deviation; 95% C.I., 95% confidence intervals.

TABLE 5 | Metabolic profiling in two children with hepatic GSDs.

Case	Sample (time)	CGM Glucose (mmol/l) Reference value: –	Lactate (mmol/l) Reference value: < 2.2	BHB (mmol/l) Reference value: < 0.42	ALT (U/l) Reference value: < 35	AST (U/l) Reference value: < 45	Cholesterol (mmol/l) Reference value: < 5.2	Triglycerides (mmol/l) Reference value: < 2.0
1	A (14:09h)	5.1	5.4	0.06	1,220	1,503	2.9	7.54
	B (16:25h)	4.8	3.7	0.08	–	–	2.8	5.85
2	C (14:19h)	5.6	4.2	0.17	217	236	6.8	9.10
	D (06:30h)	4.2	–	0.2	–	–	–	–
	E (08:43h)	4.0	1.4	0.4	337	578	6.6	9.05
	F (11:00h)	3.9	–	1.3	–	–	–	–
	G (14:00h)	3.3	1.3	1.8	–	–	6.7	3.95

Case 1 was referred by the general pediatrician and seen at the metabolic outpatient clinic at the age of eight months with failure to thrive and suspicion of hepatomegaly. Sample A was randomly obtained two hours after the last meal and sample B was obtained pre-prandially. Subsequently, he was admitted at the ward and dietary management was titrated, guided by continuous glucose monitoring (CGM) and point-of-care (POCT) beta-hydroxybutyrate (BHB) measurements. Homozygosity for the c.4529dupA, p.Tyr1510 AGL variant confirmed the diagnosis GSD IIIa. Case 2 was referred by the general pediatrician and seen at the metabolic outpatient clinic on a Friday afternoon at the age of 3.3 years with protruding abdomen, hepatomegaly, and abnormal transaminase values. The initial history did neither indicate severe metabolic decompensations, nor fasting intolerance. Sample C was randomly collected, and the boy went home with his parents, while a food diary, CGM and POCT BHB measurements were started at home over the weekend. When he returned on Monday, the food diary documented that the patient drank milk and ate meals at night times (02:10h; 03:02h) and early in the morning (05:50h). At home, his POCT glucose and BHB concentrations were 3.7 mmol/l and 1.8 mmol/l respectively, after a pause 7h47min at night. At the ward after breakfast (sample D), during real-time monitoring with an alarming CGM and glucose/BHB POCT measurements (samples E and F), sample G was obtained pre-prandially. Subsequently, dietary management was titrated and homozygosity for the c.2126T>C, p.Leu709Pro PYGL variant confirmed the diagnosis GSD VI.–, not available.*

portable ketone meter may be used in children undergoing parallel diagnostic and therapeutic metabolic profiling for hypoglycemic disorders [(52) and **Table 5**]. A hybrid protocol combining the exploration of fasting tolerance (by CGM) with so-called *in vivo* metabolic profiling (BHB and possibly additional biochemical parameters) may replace the historical approach in patients suspected with hepatic GSDs. Such approach will likely further decrease the use of diagnostic *in vivo* fasting tests. Indeed, the combination of information collected through different testing approaches will likely represent the future strategy to characterize childhood hypoglycemia, including both the diagnostic and monitoring phase. Additional factors (e.g., experience of the medical team, patients' needs, available resources, insurance policy) may result in variations in local practice, even between specialized centers.

STABLE ISOTOPE TRACING TO CHARACTERIZE CHILDHOOD HYPOGLYCEMIA

The flow of metabolites through a (disrupted) metabolic pathway is determined by both the rate of supply of substrates and the residual activities of the enzymes in the pathway. In patients with IMDs, changes in the distribution of fluxes occur secondary to enzyme defect(s) and/or altered substrate availability. By assessing the flow of metabolites through specific metabolic pathways *in vivo*, fluxomics can provide an overall description of tissue phenotypes in a relatively simple manner (53). (Radioactive or stable) isotope tracers have been used since 1930s to characterize such changes in cellular and whole-body metabolism (54). Stable isotopes techniques are well established

methods in fundamental/translational research to assess metabolic fluxes/changes (55, 56). Proof-of-concept research has also shown the potential clinical (research and care) application of stable isotopes to characterize the biochemical and clinical phenotype of IMDs associated with hypoglycemia (57, 58). An overview on current evidence paving the path for future use of stable isotope techniques in diagnosis and monitoring of IMDs characterized by hypoglycemia is provided, with specific focus on GSD I.

Principles of Stable Isotopes Methods

The core assumption of isotopic methods is that a tracer [i.e., a substance that can be administered in order to follow a natural substance (tracee)] is metabolically indistinguishable from the tracee (59). Ideally, the amount of the administered tracer is sufficiently low to avoid any effect on the physiological process investigated (59). Flux calculations are often performed when the system is in a steady state (i.e., a condition in which the tracer is infused continuously and its dilution in the analyzed compartment (e.g., plasma) remains constant over time). In this condition, blood samples are collected before the tracer intravenous infusion (after a priming dose) and several times after achieving the steady state (60).

Two types of tracers can be used: those that contain radioisotopes or those that contain stable isotopes. Radioisotopes are isotopes which release radiation as a by-product of their decay. The energy released during the decay can be measured, following the path of the tracer from reactants to products. Although such tracers have been widely used up to the 1970s (61), the health risk associated with radioactive tracers in humans and the development of affordable mass spectrometers led to the greater use of stable isotope tracers (62). Stable isotopes do not emit radiation and can be easily detected by mass spectrometry. Stable isotope tracers employed to study carbohydrate metabolism usually contain a hydrogen isotope (e.g., D-[6,6-$^{2}H_2$]-glucose, or D-[1-^{2}H]- galactose) or a carbon isotope (e.g., uniformly labelled glucose D-[U-^{13}C]-glucose or [2-^{13}C]- glycerol). Since tracer loss without net conversion to product, that may be due to recycling (for example in case of recycling of D-[2-$^{2}H_2$]-glucose by conversion of glucose-6-phosphate to fructose-6-phosphate and back), may occur at specific metabolic steps, appropriate selection of the tracer is crucial for accurate analysis (63). The use of D-[6,6-$^{2}H_2$]-glucose or D-[U-^{13}C]-glucose may in part overcome the pitfalls associated with other tracers (64). Given that each stable isotope is naturally found in the environment and in the body at a low concentration, it is important to take into account its natural abundance in order to determine the degree of tracer enrichment (65). Also, it is necessary that the degree of enrichment is high enough to be measured. The enrichment is calculated as a fraction: [tracer]/([tracer]+[tracee]) and is usually expressed as mole percent excess (MPE) (63).

For glucose metabolism during fasting, at biological steady state the rate of appearance of glucose into the circulation (R_a) represents endogenous glucose production (EGP) in the absence of an additional input of glucose. Moreover, at this steady state R_a equals the rate of glucose disposal (R_d). Experimentally, R_a is estimated during a continuous infusion of a solution of labeled glucose from the measured dilution of stable isotope labeled glucose (for example D-[U-^{13}C]-glucose) in the pool of plasma glucose at isotopic steady state. In general, the rate of infusion of labeled glucose (I) cannot be neglected in comparison with R_a. Therefore, during a stable isotope tracer infusion experiment the total rate of appearance of glucose (R_t) into the circulation is considered as, the sum of R_a and I: $R_t = R_a + I = R_d$. At isotopic steady state, the R_t can be calculated from the dilution of infused labelled glucose in the plasma glucose pool, as the ratio of fractional isotopologue enrichment of infusate over the fractional isotopologue enrichment of blood glucose, multiplied with the infusion rate. When D-[U-^{13}C]-glucose, with 6 ^{13}C carbon atoms, is applied as a glucose label $R_t = (M^{+6}_{\text{infusate}}/M^{+6}_{\text{blood glucose}}) * I$. Then R_a can be calculated as: $R_a = R_t - I = ((M^{+6}_{\text{infusate}}/M^{+6}_{\text{blood glucose}}) - 1) * I$. (59).

However, there are situations in which additional variables play a role and in which tracer enrichment can vary (non-steady state). For example, inflow of glucose (e.g., from the intestine in the post-absorptive state, or additional glucose infusion) or hormonal response (e.g., insulin, cortisol) drives glucose through different compartments before entering (and possibly re-entering) the bloodstream. Such complex conditions can still be simplified to a single-compartment model by including a pool volume of the total blood glucose pool and the changes in the [tracer]/([tracer]+[tracee]) enrichments over time in the calculations (66). Still, changes in the pool volume over time and equilibration rate of the tracer between compartments can result in aberrant results when using this approach. Frequent sampling and appropriate data fitting can minimize such errors (59). Alternatively, the pool volume can be measured (e.g., by infusing two or even three different isotopes), requiring more complex experimental designs (67).

Stable Isotopes Techniques to Study Conditions Associated With Hypoglycemia

Stable isotopes have been widely used to study the *in vivo* dynamics of glucose metabolism and conditions associated with hypoglycemia. As previously mentioned, R_a and R_d are the key variables when performing tracer studies. For blood glucose, R_a reflects the EGP in fasted conditions in the absence of any exogenous input [e.g. (e.g., glucose influx from the intestine, or additional unlabeled glucose infusion]. Physiologically, two main biochemical processes, namely gluconeogenesis and glycogenolysis, account for EGP (68). Conversely, R_d represents glucose uptake from the blood by the liver and other organs, and is determined by several metabolic pathways, including glycolysis and glycogen synthesis.

In 1977, Bier et al. investigated glucose turnover by using stable isotope tracers for the first time (20). Particularly, D-[6,6-$^{2}H_2$]-glucose intravenous infusion allowed quantification of EGP in children (with either hypoglycemia or normal glucose homeostasis) and (normoglycemic) adults, revealing that 1) physiologically EGP is higher in children than adults when normalized for body weight and 2) a linear correlation between EGP and brain weight exists in children (20). This study set the stage for further in-depth research on childhood hypoglycemia assessing glucose kinetics with stable isotope

methods (69–71). D-[6,6-$^2H_2$]-glucose intravenous infusion has been used to investigate glucose kinetics in idiopathic ketotic hypoglycemia. Particularly, it has been clarified that this condition is due to impaired EGP (rather than increased glucose utilization) (72) secondary to insufficient increase in gluconeogenesis (71). In a single patient with congenital hyperinsulinism increased EGP and glycogenolysis after glucagon administration were demonstrated. Specifically, increased glucose R_a (assessed by D-[6,6-$^2H_2$]-glucose intravenous infusion) and decreased glycerol R_a [assessed by [2-^{13}C]-glycerol intravenous infusion (73)] were noted. Additionally, various techniques to estimate *in vivo* rates of gluconeogenesis and/or glycogenolysis with stable isotopes have been developed (74).

As the liver accounts for ∼80% of EGP (with the rest accounted for mainly by the kidneys), a particular interest has developed to assess glucose kinetics with stable isotopes tracers in IMDs in which hypoglycemia is mainly due to the enzyme defect in the liver. A detailed report on the use of stable isotope methodology in IMDs goes beyond the scope of the present work [see for a review (75)]. Specific results on selected IMDs associated with hypoglycemia are presented here, with a special focus on GSD I.

Flux analysis in a mouse model of medium chain acyl-CoA dehydrogenase deficiency (MCADD) has shown that pharmacological inhibition of mitochondrial fatty acid oxidation does not affect EGP (76). Insufficient increase of gluconeogenesis upon hypoglycemia has been observed in long-chain acyl-CoA dehydrogenase-deficient mice (77). Observations on a patient with propionic acidemia showed elevated carbohydrate utilization both at rest and during exercise, suggesting that these patients rely more on carbohydrates as energy source than healthy volunteers (78). Research on hereditary fructose intolerance (HFI) showed that aldolase B contributes for around 50% of the fructose to glucose conversion in healthy subjects and that children with HFI exhibit a significantly lower conversion of fructose to glucose (79).

Several studies have made use of stable isotope tracer methodology to investigate glucose kinetics in GSD I, both in rodent models (80, 81) and humans (57, 82–88). GSD I (MIM# 232200) is an inherited disorder of glycogen metabolism due to a defect in the glucose 6-phosphatase (G6Pase) system. Defects either in the catalytic subunit (G6Pase-α, *G6PC1*) or in the microsomal glucose 6-phosphate transporter (G6PT, *SLC37A4*) cause GSD Ia and GSD Ib, respectively. Impaired glycogenolysis and gluconeogenesis result in fasting intolerance with hypoglycemia, elevated lactate, metabolic acidosis and secondary metabolic derangements. Uncooked cornstarch (UCCS) or extended-release cornstarch (Glycosade®) and/or continuous gastric drip-feeding is the cornerstone of the treatment (52).

Assessing EGP in GSD I has been one of the key research objectives for decades (57, 80, 82–88). After evidence on residual EGP was first provided in two patients with GSD I (89), several studies have made use of stable isotope methods to quantify glucose production in GSD I patients (57, 82–88). Those studies showed that, despite G6Pase deficiency, a limited (up to ∼60% of normal) capacity of EGP could be found in GSD Ia. Yet, estimated EGP varied considerably among patients. In some of these studies EGP was likely overestimated due to the contribution of dietary glucose or intravenous/intragastric glucose infusions, which caused total glucose R_a to exceed EGP. The reason why there is still residual EGP in GSD I is still not clear. Various mechanisms have been proposed, including residual G6Pase activity (90), extra-hepatic/renal/intestinal EGP (91), increased debrancher enzyme (AGL) activity, and increased acid alpha glucosidase (GAA) activity (82). Studies on rodent models have contributed to further delineate the (patho) physiological basis of this process. Stable isotope tracer methodology to investigate glucose kinetics in animal models for GSD I was first applied in an acute model of GSD Ib in fasted rats using the pharmacological G6PT-inhibitor S4048. The method applied consisted of an infusion of stable isotopes D-[U-^{13}C]-glucose, [2-^{13}C]-glycerol, and D-[1-^{2}H]-galactose, as well as paracetamol, to quantify hepatic glucose and glycogen metabolism (80). Later, this same methodology was applied to mice with hepatocyte-specific *G6pc* deficiency, a model for hepatic GSD Ia (81), which retained about 30% of EGP compared to their wild-type littermates. Primary hepatocytes from these animals showed that residual glucose production (∼20% of wild-type littermates) was almost completely abolished upon treatment with an α-glucosidase inhibitor, which inhibits both AGL and GAA. This indicates that either increased glycogen debranching and/or lysosomal glycogen breakdown accounts for the production of free glucose by the GSD Ia liver and hence could explain the residual EGP in GSD I (81). Nonetheless, research assessing the utilization of (various types of) cornstarch in hepatic GSD patients (92, 93) supports the extensibility of stable isotopes methods also to human research.

DISCUSSION AND FUTURE PERSPECTIVES

Although the past years have witnessed major advances in understanding the pathophysiology of disorders associated with hypoglycemia, relevant limitations in assessment and monitoring strategies remain. The current diagnostic approach to children presenting with hypoglycemia includes, besides medical/dietary history and clinical examination, biochemical tests (performed on "critical samples") and eventually genetic confirmation (21). However, critical samples or reliable biochemical data are not always available or may not provide conclusive information. Also, traditional biochemical markers are static parameters and do not always appear sufficient to explain the phenotypic variability in patients with IMDs associated with hypoglycemia (94). Furthermore, those biomarkers may not adequately reflect the dynamic pathophysiological processes taking place in response to external factors such as metabolic stressors or treatment. Thus, there remain a group of patients with unsolved diagnostic and/or monitoring questions, that may be addressed by dynamic methods, such as *in vivo* functional tests, CGM and stable isotope tracing.

Functional *in vivo* tests have the advantage of characterizing metabolic pathways, such as glycogenolysis, gluconeogenesis, mitochondrial fatty acid oxidation, ketogenesis, and ketolysis, in a dynamic fashion. Yet, their use is progressively decreasing. Therefore, we do not recommend performing such tests in unexperienced centers. Several functional *in vivo* tests (e.g., controlled fasting challenge) are currently designed to monitor management after novel, innovative treatments (NCT03517085, NCT03665636, NCT05095727, NCT NCT04538989, NCT04720859, NCT03761693) in centers of expertise. Fasting test may still play a role in the diagnostic process of congenital hyperinsulinism. Yet, the development of safer and simpler diagnostic and monitoring tools for clinical research/care remains a compelling need (95).

CGM constitutes a highly informative technique to (non-invasively) monitor glucose levels in a dynamic fashion in patients with hypoglycemia (39–41, 45). Although physicians working in centers of expertise have become experienced with the CGM technology, its use in less experienced centers remains limited. Therefore, knowledge dissemination on the CGM technology and data interpretation is paramount. In fact, given its relatively low organizational burden and invasiveness, CGM may constitute a valuable technique. Ideally, individual patients' CGM results could be compared with (1) the patient's historical CGM data before and after intervention, and CGM data from both (2) an age-matched disease cohort and (3) age-matched healthy controls. Shah et al. recently reported CGM profiles from 153 nonpregnant, healthy, nondiabetic children and adults (age ≥6 years) with nonobese body mass index, using a blinded Dexcom G6 CGM, with once-daily calibration, for up to 10 days (96). Advanced CGM data analysis represents a promising strategy for minimally invasive patient monitoring (48). However, several challenges constrain CGM use in patients with hypoglycemia. First, neither general agreement on relevant CGM-related outcome parameters nor reference values for such parameters exist for patients with IMDs. Ideally, individual patients' CGM results would be comparable once these issues have been solved. Second, the lag time (i.e., the time delay between a change in plasma glucose and the reporting of this change by the CGM device) may affect CGM reliability in patients experiencing sudden drops in glucose concentrations, as in hepatic GSDs. Third, most CGM devices do not contain predictive alarms (i.e., predicting an upcoming low glucose event using trend information) and rely upon the user to identify hypoglycemia and make manual adjustments. Also, current predictive alarms are based on a linear function of glucose trends, which may not adequately reflect the exponential decrease observed in hypoglycemic GSD patients (97). Likely the progress in machine learning and deep learning approaches and artificial neural networks will develop reliable algorithms for hypoglycemia prediction in patients with IMDs (35). Fourth, some devices require fingerprick calibration, which currently prioritizes hyperglycemia over hypoglycemia, thus constituting a potential additional source of inaccuracy, particularly a in the low range of blood glucose (35). Notably, most recent devices such as Dexcom G6 and Freestyle Libre are factory-calibrated (98). Fifth, as no specific CGM device is formally licensed for patients with IMDs, several types of CGM devices are used (**Table 3**) presenting with different functionalities, advantages, and limitations. Additionally, data management applications currently vary, depending on the type of CGM device installed and interconnectivity between CGM software packages, and digital health records are lacking. Harmonization of data management applications as well as improved interconnectivity between CGM software packages and data health records are strongly warranted to facilitate data sharing among healthcare professionals and beyond towards citizen science applications. Sixth, while a 14-day data collection is recommended in DM patients (99), the number of measurements required to generate reliable CGM profiles in patients with IMDs is still unknown.

Stable isotope techniques appear attractive as they give access to the *in vivo* dynamics of hypoglycemia from a pathophysiological perspective. In principle, this may prevent a late diagnosis in patients presenting with milder phenotypes (100, 101). Yet, this technique has been explored experimentally in a limited number of conditions (e.g., idiopathic ketotic hypoglycemia, GSD I, congenital hyperinsulinism), but it is currently not clinically implemented, neither applied in clinical trials. Potential clinical benefit of this approach includes the definition of an easy and safe method to guide the diagnosis of patients with hypoglycemia and to improve patient monitoring, possibly constituting an additional tool to assess the effect of novel treatments (e.g., gene therapy, mRNA therapy). Given the safety of this methodology, it appears well suited for clinical trials in humans. Traditionally, venous blood samples are collected before the intravenous tracer infusion (after a priming dose) and several times after achieving the steady state (60). Such a procedure may potentially hamper the translation of this approach into clinical research/care in its current form. Previous research has shown the reliability of dried blood spot (DBS) sampling (compared to traditional venous sampling) in mice by tail bleeding to assess glucose kinetics following infusion of D-[U-^{13}C]-glucose, D-[1-^{2}H]-galactose, and [2-^{13}C]-glycerol (102) or after an intraperitoneal injection of D-[6,6-^{2}H]-glucose (58). Although these findings pave the way for possible oral (non-steady-state) administration of stable isotope tracers to humans (who are metabolically in a steady state, i.e. upon fasting without any unknown (unmeasured) and/or non-steady additional exogenous glucose input), the effect of this administration route on the tracer bioavailability remains unknown. Future studies exploring this approach in humans are warranted. Additionally, the use of stable isotope tracers in animal models can guide further dissection of pathophysiological derangements occurring in IMDs.

In addition to functional *in vivo* tests, metabolic profiling by CGM and stable isotope techniques, the microdialysis technique, at least in theory, would enable continuous sampling of multiple metabolites from the extracellular space from various tissues, such as blood, adipose tissue, or brain in experimental models (103, 104). The combination of stable isotope tracing and microdialysis in animal models appears intriguing (105). As microdialysis allows not only glucose monitoring (106, 107) but also sampling of other metabolites (e.g., glycerol, acylcarnitines) (108), it has a great potential for experimental

research in patients with IMDs. Still, there are currently no commercial systems available.

AUTHOR CONTRIBUTIONS

AR, MGSR, MHO and TGJD wrote the first version of the manuscript. THvD, BMB, D-JR and TGJD critically reviewed the manuscript. AR drafted and wrote the final version of manuscript. TGJD critically reviewed the final version of the manuscript. All authors substantially contributed to the work and were involved in (a) conception and design of the study and/or analysis and interpretation of data, and (b) revising the article critically for important intellectual content. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. All authors confirm the absence of previous similar or simultaneous publications. All authors contributed to the article and approved the submitted version.

REFERENCES

1. Gandhi K. Approach to Hypoglycemia in Infants and Children. *Transl Pediatr* (2017) 6(4):408–20. doi: 10.21037/tp.2017.10.05
2. Cornblath M, Hawdon JM, Williams AF, Aynsley-Green A, Ward-Platt MP, Schwartz R, et al. Controversies Regarding Definition of Neonatal Hypoglycemia: Suggested Operational Thresholds. *Pediatrics* (2000) 105 (5):1141–5. doi: 10.1542/peds.105.5.1141
3. Thornton PS, Stanley CA, De Leon DD, Harris D, Haymond MW, Hussain K, et al. Recommendations From the Pediatric Endocrine Society for Evaluation and Management of Persistent Hypoglycemia in Neonates, Infants, and Children. *J Pediatr* (2015) 167(2):238–45. doi: 10.1016/j.jpeds.2015.03.057
4. Kallem VR, Pandita A, Gupta G. Hypoglycemia: When to Treat? *Clin Med Insights Pediatr* (2017) 11:1179556517748913. doi: 10.1177/1179556517748913
5. Suh SW, Hamby AM, Swanson RA. Hypoglycemia, Brain Energetics, and Hypoglycemic Neuronal Death. *Glia* (2007) 55(12):1280–6. doi: 10.1002/glia.20440
6. Suh SW, Aoyama K, Chen Y, Garnier P, Matsumori Y, Gum E, et al. Hypoglycemic Neuronal Death and Cognitive Impairment are Prevented by Poly(ADP-Ribose) Polymerase Inhibitors Administered After Hypoglycemia. *J Neurosci* (2003) 23(33):10681–90. doi: 10.1523/JNEUROSCI.23-33-10681.2003
7. McKinlay CJ, Alsweiler JM, Ansell JM, Anstice NS, Chase JG, Gamble GD, et al. Neonatal Glycemia and Neurodevelopmental Outcomes at 2 Years. *N Engl J Med* (2015) 373(16):1507–18. doi: 10.1056/NEJMoa1504909
8. Tas E, Garibaldi L, Muzumdar R. Glucose Homeostasis in Newborns: An Endocrinology Perspective. *Neoreviews* (2020) 21(1):e14–29. doi: 10.1542/neo.21-1-e14
9. Saudubray JM, van den Berghe V, Walter J. (2016). *Inherited Metabolic Diseases. 6th Edition*. (Berlin, Germany: Springer).
10. Ghosh A, Banerjee I, Morris AAM. Recognition, Assessment and Management of Hypoglycaemia in Childhood. *Arch Dis Child* (2016) 101 (6):575–80. doi: 10.1136/archdischild-2015-308337
11. Chaussain JL, Georges P, Calzada L, Job JC. Glycemic Response to 24-Hour Fast in Normal Children: III. Influence of Age. *J Pediatr* (1977) 91(5):711–4. doi: 10.1016/s0022-3476(77)81020-2
12. Haymond MW, Karl IE, Clarke WL, Pagliara AS, Santiago JV. Differences in Circulating Gluconeogenic Substrates During Short-Term Fasting in Men, Women, and Children. *Metabolism* (1982) 31(1):33–42. doi: 10.1016/0026-0495(82)90024-5
13. Lamers KJ, Doesburg WH, Gabreëls FJ, Romsom AC, Renier WO, Wevers RA, et al. Reference Values of Blood Components Related to Fuel Metabolism in Children After an Overnight Fast. *Clin Chim Acta* (1985) 145(1):17–26. doi: 10.1016/0009-8981(85)90015-4
14. Lamers KJ, Doesburg WH, Gabreëls FJ, Lemmens WA, Romsom AC, Wevers RA, et al. The Concentration of Blood Components Related to Fuel Metabolism During Prolonged Fasting in Children. *Clin Chim Acta* (1985) 152(1-2):155–63. doi: 10.1016/0009-8981(85)90186-X
15. Bonnefont JP, Specola NB, Vassault A, Lombes A, Ogier H, de Klerk JB, et al. The Fasting Test in Paediatrics: Application to the Diagnosis of Pathological Hypo- and Hyperketotic States. *Eur J Pediatr* (1990) 150(2):80–5. doi: 10.1007/BF02072043
16. van Veen MR, van Hasselt PM, de Sain-van der Velden MG, Verhoeven N, Hofstede FC, de Koning TJ, et al. Metabolic Profiles in Children During Fasting. *Pediatrics* (2011) 127(4):e1021–7. doi: 10.1542/peds.2010-1706
17. Sunehag A, Ewald U, Larsson A, Gustafsson J. Glucose Production Rate in Extremely Immature Neonates (< 28 Weeks) Studied by Use of Deuterated Glucose. *Pediatr Res* (1993) 33(2):97–100. doi: 10.1203/00006450-199302000-00001
18. Bier DM, Leake RD, Haymond MW, Arnold KJ, Gruenke LD, Sperling MA, et al. Measurement of "True" Glucose Production Rates in Infancy and Childhood With 6,6-Dideuteroglucose. *Diabetes* (1977) 26(11):1016–23. doi: 10.2337/diab.26.11.1016
19. Huidekoper HH, Ackermans MT, Ruiter A, Sauerwein HP, Wijburg FA. Endogenous Glucose Production From Infancy to Adulthood: A non-Linear Regression Model. *Arch Dis Child* (2014) 99(12):1098–102. doi: 10.1136/archdischild-2013-305718
20. Bougnères PF, Artavia-Loria E, Henry S, Basdevant A, Castaño L. Increased Basal Glucose Production and Utilization in Children With Recent Obesity Versus Adults With Long-Term Obesity. *Diabetes* (1989) 38(4):477–83. doi: 10.2337/diab.38.4.477
21. Casertano A, Rossi A, Fecarotta S, Rosanio FM, Moracas C, Di Candia F, et al. An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use. *Front Endocrinol (Lausanne)* (2021) 12:684011. doi: 10.3389/fendo.2021.684011
22. Haymond MW, Pagliara AS. Ketotic Hypoglycaemia. *Clin Endocrinol Metab* (1983) 12(2):447–62. doi: 10.1016/S0300-595X(83)80051-6
23. Drachmann D, Hoffmann E, Carrigg A, Davis-Yates B, Weaver V, Thornton P, et al. Towards Enhanced Understanding of Idiopathic Ketotic Hypoglycemia: A Literature Review and Introduction of the Patient Organization, Ketotic Hypoglycemia International. *Orphan J Rare Dis* (2021) 16(1):173. doi: 10.1186/s13023-021-01797-2
24. Fernandes J, Huijing F, van de Kamer JH. A Screening Method for Liver Glycogen Diseases. *Arch Dis Child* (1969) 44(235):311–7. doi: 10.1136/adc.44.235.311
25. Fernandes J, Van De Kamer JH. Studies on the Utilization of Hexoses in Liver Glycogen Disease. *Pediatrics* (1965) 35:470–7. doi: 10.1542/peds.35.3.470
26. Fernandes J, van de Kamer JH. Hexose and Protein Tolerance Tests in Children With Liver Glycogenosis Caused by a Deficiency of the Debranching Enzyme System. *Pediatrics* (1968) 41(5):935–44. doi: 10.1542/peds.41.5.935
27. N Blau, C Dionisi-Vici, CR Ferreira, C Vianey-Saban and CDM van Karnebeek eds. *Physician's Guide to the Diagnosis, Treatment, and Follow-Up of Inherited Metabolic Diseases*. Berlin Heidelberg: Springer-Verlag (2022).
28. Cahill GFJr. President's Address. Starvation. *Trans Am Clin Climatol Assoc* (1983) 94:1–21.
29. Morris AA, Thekekara A, Wilks Z, Clayton PT, Leonard JV, Aynsley-Green A. Evaluation of Fasts for Investigating Hypoglycaemia or Suspected Metabolic Disease. *Arch Dis Child* (1996) 75(2):115–9. doi: 10.1136/adc.75.2.115
30. Bappal B, Mula-Abed WA. Evaluation of Diagnostic Fasting in the Investigation of Hypoglycemia in Children Omani Experience. *Oman Med J* (2007) 22(3):36–41.

31. Graves LE, Stewart K, Ambler GR, Bhattacharya K, Srinivasan S. Investigating Paediatric Hypoglycaemia: Dynamic Studies at a Tertiary Paediatric Hospital. *J Paediatr Child Health* (2021) 57(6):888–93. doi: 10.1111/jpc.15349
32. Demirbilek H, Hussain K. Congenital Hyperinsulinism: Diagnosis and Treatment Update. *J Clin Res Pediatr Endocrinol* (2017) 9(Suppl 2):69–87. doi: 10.4274/jcrpe.2017.S007
33. Rosenfeld E, Ganguly A, De Leon DD. Congenital Hyperinsulinism Disorders: Genetic and Clinical Characteristics. *Am J Med Genet C Semin Med Genet* (2019) 181(4):682–92. doi: 10.1002/ajmg.c.31737
34. Karam JH, Lorenzi M, Young CW, Burns AD, Prosser PR, Grodsky GM, et al. Feedback-Controlled Dextrose Infusion During Surgical Management of Insulinomas. *Am J Med* (1979) 66(4):675–80. doi: 10.1016/0002-9343(79)91183-5
35. Worth C, Dunne M, Ghosh A, Harper S, Banerjee I. Continuous Glucose Monitoring for Hypoglycaemia in Children: Perspectives in 2020. *Pediatr Diab* (2020) 21(5):697–706. doi: 10.1111/pedi.13029
36. American Diabetes Association. Standards of Medical Care in Diabetes. *Diabetes Care* (2020) 43:S1–2. doi: 10.2337/dc20-S002
37. Takahashi H, Iwahashi N, Kirigaya J, Kataoka S, Minamimoto Y, Gohbara M, et al. Glycemic Variability Determined With a Continuous Glucose Monitoring System can Predict Prognosis After Acute Coronary Syndrome. *Cardiovasc Diabetol* (2018) 17(1):116. doi: 10.1186/s12933-018-0761-5
38. Jamshed H, Beyl RA, Della Manna DL, Yang ES, Ravussin E, Peterson CM. Early Time-Restricted Feeding Improves 24-Hour Glucose Levels and Affects Markers of the Circadian Clock, Aging, and Autophagy in Humans. *Nutrients* (2019) 11(6):1234. doi: 10.3390/nu11061234
39. Cambiaso P, Schiaffini R, Pontrelli G, Carducci C, Ubertini G, Crea F, et al. Nocturnal Hypoglycaemia in ACTH and GH Deficient Children: Role of Continuous Glucose Monitoring. *Clin Endocrinol (Oxf)* (2013) 79(2):232–7. doi: 10.1111/cen.12123
40. Alsaffar H, Turner L, Yung Z, Didi M, Senniappan S. Continuous Flash Glucose Monitoring in Children With Congenital Hyperinsulinism; First Report on Accuracy and Patient Experience. *Int J Pediatr Endocrinol* (2018) 2018:3. doi: 10.1186/s13633-018-0057-2
41. Morales-Alvarez MC, Ricardo-Silgado ML, Lemus HN, González-Devia D, Mendivil CO. Fructosuria and Recurrent Hypoglycemia in a Patient With a Novel c.1693T>A Variant in the 3' Untranslated Region of the Aldolase B Gene. *SAGE Open Med Case Rep* (2019) 7:2050313X18823098. doi: 10.1177/2050313X18823098
42. Hershkovitz E, Rachmel A, Ben-Zaken H, Phillip M. Continuous Glucose Monitoring in Children With Glycogen Storage Disease Type I. *J Inherit Metab Dis* (2001) 24(8):863–9. doi: 10.1023/A:1013996325720
43. Maran A, Crepaldi C, Avogaro A, Catuogno S, Burlina A, Poscia A, et al. Continuous Glucose Monitoring in Conditions Other Than Diabetes. *Diabetes Metab Res Rev* (2004) 20:S50–5. doi: 10.1002/dmrr.518
44. White F, Jones SA. The Use of Continuous Glucose Monitoring in the Practical Management of Glycogen Storage Disorders. *J Inherit Metab Dis* (2011) 34:631–642.25. doi: 10.1007/s10545-011-9335-3
45. Kasapkara ÇS, Cinasal Demir G, Hasanoglu A, Tümer L. Continuous Glucose Monitoring in Children With Glycogen Storage Disease Type I. *Eur J Clin Nutr* (2014) 68:101–5. doi: 10.1038/ejcn.2013.186
46. Herbert M, Pendyal S, Rairikar MR, Halaby C, Benjamin RW, Kishnani PS. Role of Continuous Glucose Monitoring in the Management of Glycogen Storage Dis-Orders. *J Inherit Metab Dis* (2018) 41:917–27. doi: 10.1007/s10545-018-0200-5
47. Kaiser N, Gautschi M, Bosanska L, Meienberg F, Baumgartner MR, Spinas GA, et al. Glycemic Control and Complications in Glycogen Storage Disease Type I: Results From the Swiss Registry. *Mol Gen Metab* (2019) 126:355–61. doi: 10.1016/j.ymgme.2019.02.008
48. Peeks F, Hoogeveen IJ, Feldbrugge RL, Burghard R, de Boer F, Fokkert-Wilts MJ, et al. A Retrospective in-Depth Analysis of Continuous Glucose Monitoring Datasets for Patients With Hepatic Glycogen Storage Disease: Recommended Outcome Parameters for Glucose Management. *J Inherit Metab Dis* (2021) 44(5):1136–50. doi: 10.1002/jimd.12383
49. Rossi A, Miele E, Fecarotta S, Veiga-da-Cunha M, Martinelli M, Mollica C, et al. Crohn Disease-Like Enterocolitis Remission After Empagliflozin Treatment in a Child With Glycogen Storage Disease Type Ib: A Case Report. *Ital J Pediatr* (2021) 47(1):149. doi: 10.1186/s13052-021-01100-w
50. Wolfsdorf JI, Sadeghi-Nejad A, Senior B. Fat-Derived Fuels During a 24-Hour Fast in Children. *Eur J Pediatr* (1982) 138(2):141–4. doi: 10.1007/BF00441141
51. Parmar K, Mosha M, Weinstein DA, Riba-Wolman R. Method Comparison of Beta-Hydroxybutyrate Point-of-Care Testing to Serum in Healthy Children. *JIMD Rep* (2021) 62(1):85–90. doi: 10.1002/jmd2.12245
52. Weinstein DA, Steuerwald U, De Souza CFM, Derks TGJ. Inborn Errors of Metabolism With Hypoglycemia: Glycogen Storage Diseases and Inherited Disorders of Gluconeogenesis. *Pediatr Clin North Am* (2018) 65(2):247–65. doi: 10.1016/j.pcl.2017.11.005
53. Klein S, Heinzle E. Isotope Labeling Experiments in Metabolomics and Fluxomics. *Wil Interdiscip Rev Syst Biol Med* (2012) 4(3):261–72. doi: 10.1002/wsbm.1167
54. Schoenheimer R, Rittenberg D. The APPLICATION of ISOTOPES to THE Study OF Intermediary Metabolism. *Science* (1938) 87(2254):221–6. doi: 10.1126/science.87.2254.221
55. Wilkinson DJ. Historical and Contemporary Stable Isotope Tracer Approaches to Studying Mammalian Protein Metabolism. *Mass Spectrom Rev* (2018) 37(1):57–80. doi: 10.1002/mas.21507
56. Yuan M, Kremer DM, Huang H, Breitkopf SB, Ben-Sahra I, Manning BD, et al. *Ex Vivo* and *In Vivo* Stable Isotope Labelling of Central Carbon Metabolism and Related Pathways With Analysis by LC-MS/MS. *Nat Protoc* (2019) 14(2):313–30. doi: 10.1038/s41596-018-0102-x
57. Huidekoper HH, Visser G, Ackermans MT, Sauerwein HP, Wijburg FA. A Potential Role for Muscle in Glucose Homeostasis: In Vivo Kinetic Studies in Glycogen Storage Disease Type 1a and fructose-1,6-bisphosphatase Deficiency. *J Inherit Metab Dis* (2010) 33(1):25–31. doi: 10.1007/s10545-009-9030-9
58. van Dijk TH, Laskewitz AJ, Grefhorst A, Boer TS, Bloks VW, Kuipers F, et al. A Novel Approach to Monitor Glucose Metabolism Using Stable Isotopically Labelled Glucose in Longitudinal Studies in Mice. *Lab Anim* (2013) 47(2):79–88. doi: 10.1177/0023677212473714
59. Cobelli C, Foster DM, Toffolo GM. *Tracer Kinetics in Biomedical Research*. New York: Kluwer Academic/Plenum Publishers (2000).
60. Kim IY, Suh SH, Lee IK, Wolfe RR. Applications of Stable, Nonradioactive Isotope Tracers in In Vivo Human Metabolic Research. *Exp Mol Med* (2016) 48(1):e203. doi: 10.1038/emm.2015.97
61. Wolfe RR. *Radioactive and Stable Isotope Tracers in Biomedicine. 1st ed*. New York, NY, USA: Wiley-Liss, Inc (1992).
62. Umpleby M, Fielding BA. Stable Isotopes in Nutrition Research. *Nutr Res Methodol* (2015) 2015:250–64. doi: 10.1002/9781119180425.ch17
63. Wolfe RR, Chinkes DL. Isotopic Tracers in Metabolic Research. In: *Principles and Practice of Kinetic Analysis*. Hoboken, NJ: Wiley-Liss (2005).
64. Wajngot A, Chandramouli V, Schumann WC, Kumaran K, Efendić S, Landau BR. Testing of the Assumptions Made in Estimating the Extent of Futile Cycling. *Am J Physiol* (1989) 256(5 Pt 1):E668–75. doi: 10.1152/ajpendo.1989.256.5.E668
65. Davies PSW. Stable Isotopes: Their Use and Safety in Human Nutrition Studies. *Eur J Clin Nutr* (2020) 74(3):362–5. doi: 10.1038/s41430-020-0580-0
66. Vella A, Rizza RA. Application of Isotopic Techniques Using Constant Specific Activity or Enrichment to the Study of Carbohydrate Metabolism. *Diabetes* (2009) 58(10):2168–74. doi: 10.2337/db09-0318
67. Finegood DT, Bergman RN, Vranic M. Estimation of Endogenous Glucose Production During Hyperinsulinemic-Euglycemic Glucose Clamps. Comparison of Unlabeled and Labeled Exogenous Glucose Infusates. *Diabetes* (1987) 36(8):914–24. doi: 10.2337/diab.36.8.914
68. Sharabi K, Tavares CD, Rines AK, Puigserver P. Molecular Pathophysiology of Hepatic Glucose Production. *Mol Aspects Med* (2015) 46:21–33. doi: 10.1016/j.mam.2015.09.003
69. Schwartz NS, Clutter WE, Shah SD, Cryer PE. Glycemic Thresholds for Activation of Glucose Counterregulatory Systems are Higher Than the Threshold for Symptoms. *J Clin Invest* (1987) 79(3):777–81. doi: 10.1172/JCI112884
70. Hawdon JM, Aynsley-Green A, Bartlett K, Ward Platt MP. The Role of Pancreatic Insulin Secretion in Neonatal Glucoregulation. II. Infants With

Disordered Blood Glucose Homoeostasis. *Arch Dis Child* (1993) 68(3):280–5. doi: 10.1136/adc.68.3_spec_no.280
71. Huidekoper HH, Duran M, Turkenburg M, Ackermans MT, Sauerwein HP, Wijburg FA. Fasting Adaptation in Idiopathic Ketotic Hypoglycemia: A Mismatch Between Glucose Production and Demand. *Eur J Pediatr* (2008) 167(8):859–65. doi: 10.1007/s00431-007-0598-5
72. Bodamer OA, Hussein K, Morris AA, Langhans CD, Rating D, Mayatepek E, et al. Glucose and Leucine Kinetics in Idiopathic Ketotic Hypoglycaemia. *Arch Dis Child* (2006) 91(6):483–6. doi: 10.1136/adc.2005.089425
73. Cederblad F, Ewald U, Gustafsson J. Effect of Glucagon on Glucose Production, Lipolysis, and Gluconeogenesis in Familial Hyperinsulinism. *Horm Res* (1998) 50(2):94–8. doi: 10.1159/000023242
74. Chung ST, Chacko SK, Sunehag AL, Haymond MW. Measurements of Gluconeogenesis and Glycogenolysis: A Methodological Review. *Diabetes* (2015) 64(12):3996–4010. doi: 10.2337/db15-0640
75. Reijngoud DJ. Flux Analysis of Inborn Errors of Metabolism. *J Inherit Metab Dis* (2018) 41(3):309–28. doi: 10.1007/s10545-017-0124-5
76. Derks TG, van Dijk TH, Grefhorst A, Rake JP, Smit GP, Kuipers F, et al. Inhibition of Mitochondrial Fatty Acid Oxidation In Vivo Only Slightly Suppresses Gluconeogenesis But Enhances Clearance of Glucose in Mice. *Hepatology* (2008) 47(3):1032–42. doi: 10.1002/hep.22101
77. Houten SM, Herrema H, Te Brinke H, Denis S, Ruiter JP, van Dijk TH, et al. Impaired Amino Acid Metabolism Contributes to Fasting-Induced Hypoglycemia in Fatty Acid Oxidation Defects. *Hum Mol Genet* (2013) 22 (25):5249–61. doi: 10.1093/hmg/ddt382
78. Storgaard JH, Madsen KL, Løkken N, Vissing J, van Hall G, Lund AM, et al. Impaired Lipolysis in Propionic Acidemia: A New Metabolic Myopathy? *JIMD Rep* (2020) 53(1):16–21. doi: 10.1002/jmd2.12113
79. Gopher A, Vaisman N, Mandel H, Lapidot A. Determination of Fructose Metabolic Pathways in Normal and Fructose-Intolerant Children: A 13C NMR Study Using [U-13C]Fructose. *Proc Natl Acad Sci USA* (1990) 87 (14):5449–53. doi: 10.1073/pnas.87.14.5449
80. van Dijk TH, van der Sluijs FH, Wiegman CH, Baller JF, Gustafson LA, Burger HJ, et al. Acute Inhibition of Hepatic glucose-6-phosphatase Does Not Affect Gluconeogenesis But Directs Gluconeogenic Flux Toward Glycogen in Fasted Rats. A Pharmacological Study With the Chlorogenic Acid Derivative S4048. *J Biol Chem* (2001) 276(28):25727–35. doi: 10.1074/jbc.M101223200
81. Hijmans BS, Boss A, van Dijk TH, Soty M, Wolters H, Mutel E, et al. Hepatocytes Contribute to Residual Glucose Production in a Mouse Model for Glycogen Storage Disease Type Ia. *Hepatology* (2017) 66(6):2042–54. doi: 10.1002/hep.29389
82. Tsalikian E, Simmons P, Gerich JE, Howard C, Haymond MW. Glucose Production and Utilization in Children With Glycogen Storage Disease Type I. *Am J Physiol* (1984) 247(4 Pt 1):513. doi: 10.1152/ajpendo.1984.247.4.E513
83. Schwenk WF, Haymond MW. Optimal Rate of Enteral Glucose Administration in Children With Glycogen Storage Disease Type I. *N Engl J Med* (1986) 314(11):682–5. doi: 10.1056/NEJM198603133141104
84. Kalderon B, Korman SH, Gutman A, Lapidot A. Glucose Recycling and Production in Glycogenosis Type I and III: Stable Isotope Technique Study. *Am J Physiol Endocrinol Metab* (1989) 257(3):E346–53. doi: 10.1152/ajpendo.1989.257.3.E346
85. Kalderon B, Korman SH, Gutman A, Lapidot A. Estimation of Glucose Carbon Recycling in Children With Glycogen Storage Disease: A13c NMR Study Using [U-13C]Glucose. *Proc Natl Acad Sci USA* (1989) 86:4690–4. doi: 10.1073/pnas.86.12.4690
86. Collins JE, Bartlett K, Leonard JV, Aynsley-Green A. Glucose Production Rates in Type 1 Glycogen Storage Disease. *J Inherit Metab Dis* (1990) 13 (2):195–206. doi: 10.1007/BF01799686
87. Rother KI, Schwenk WF. Glucose Production in Glycogen Storage Disease I is Not Associated With Increased Cycling Through Hepatic Glycogen. *Am J Physiol* (1995) 269(4 Pt 1):774. doi: 10.1152/ajpendo.1995.269.4.E774
88. Weghuber D, Mandl M, Krssák M, Roden M, Nowotny P, Brehm A, et al. Characterization of Hepatic and Brain Metabolism in Young Adults With Glycogen Storage Disease Type 1: A Magnetic Resonance Spectroscopy Study. *Am J Physiol Endocrinol Metab* (2007) 293(5):E1378–84. doi: 10.1152/ajpendo.00658.2006
89. Powell RC, Wentworth SM, Brandt IK. Endogenous Glucose Production in Type I Glycogen Storage Disease. *Metabolism* (1981) 30(5):443–50. doi: 10.1016/0026-0495(81)90178-5
90. Spencer-Peet J, Norman ME, Lake BD, McNamara J, Patrick AD. Hepatic Glycogen Storage Disease. *Clin Lab Findings 23 Cases Q J Med* (1971) 40 (157):95–114.
91. Shieh JJ, Pan CJ, Mansfield BC, Chou JY. A glucose-6-phosphate Hydrolase, Widely Expressed Outside the Liver, can Explain Age-Dependent Resolution of Hypoglycemia in Glycogen Storage Disease Type Ia. *J Biol Chem* (2003) 278(47):47098–103. doi: 10.1074/jbc.M309472200
92. Bodamer OA, Feillet F, Lane RE, Lee PJ, Dixon MA, Halliday D, et al. Utilization of Cornstarch in Glycogen Storage Disease Type Ia. *Eur J Gastroenterol Hepatol* (2002) 14(11):1251–6. doi: 10.1097/00042737-200211000-00014
93. Bhattacharya K, Orton RC, Qi X, Mundy H, Morley DW, Champion MP, et al. A Novel Starch for the Treatment of Glycogen Storage Diseases. *J Inherit Metab Dis* (2007) 30(3):350–7. doi: 10.1007/s10545-007-0479-0
94. Peeks F, Steunenberg TAH, de Boer F, Rubio-Gozalbo ME, Williams M, Burghard R, et al. Clinical and Biochemical Heterogeneity Between Patients With Glycogen Storage Disease Type IA: The Added Value of CUSUM for Metabolic Control. *J Inherit Metab Dis* (2017) 40(5):695–702. doi: 10.1007/s10545-017-0039-1
95. Derks TGJ, Rodriguez-Buritica DF, Ahmad A, de Boer F, Couce ML, Grünert SC, et al. Glycogen Storage Disease Type Ia: Current Management Options, Burden and Unmet Needs. *Nutrients* (2021) 13 (11):3828. doi: 10.3390/nu13113828
96. Shah VN, DuBose SN, Li Z, Beck RW, Peters AL, Weinstock RS, et al. Continuous Glucose Monitoring Profiles in Healthy Nondiabetic Participants: A Multicenter Prospective Study. *J Clin Endocrinol Metab* (2019) 104(10):4356–64. doi: 10.1210/jc.2018-02763
97. Rodbard D. Continuous Glucose Monitoring: A Review of Successes, Challenges, and Opportunities. *Diabetes Technol Ther* (2016) 18(Suppl 2): S3–S13. doi: 10.1089/dia.2015.0417
98. Dexcom, Inc. *Make Knowledge Your Superpower With Thedexcom G6®CGM System*. San Diego, CA: Dexcom, Inc (2019). Available at: https://www.dexcom.com/g6-cgm-system.
99. Ajjan R, Slattery D, Wright E. Continuous Glucose Monitoring: A Brief Review for Primary Care Practitioners. *Adv Ther* (2019) 36(3):579–96. doi: 10.1007/s12325-019-0870-x
100. Cassiman D, Libbrecht L, Verslype C, Meersseman W, Troisi R, Zucman-Rossi J, et al. An Adult Male Patient With Multiple Adenomas and a Hepatocellular Carcinoma: Mild Glycogen Storage Disease Type Ia. *J Hepatol* (2010) 53(1):213–7. doi: 10.1016/j.jhep.2010.03.002
101. Moest W, van der Deure W, Koster T, Spee-Dropková M, Swart-Busscher L, de Haas RJ, et al. Glycogen Storage Disease Type Ia: Adult Presentation With Microcytic Anemia and Liver Adenomas. *Hepatology* (2018) 68(2):780–2. doi: 10.1002/hep.29858
102. van Dijk TH, Boer TS, Havinga R, Stellaard F, Kuipers F, Reijngoud DJ. Quantification of Hepatic Carbohydrate Metabolism in Conscious Mice Using Serial Blood and Urine Spots. *Anal Biochem* (2003) 322(1):1–13. doi: 10.1016/j.ab.2003.07.008
103. Arner P, Bolinder J. Microdialysis of Adipose Tissue. *J Intern Med* (1991) 230 (4):381–6. doi: 10.1111/j.1365-2796.1991.tb00461.x
104. Young B, Kalanuria A, Kumar M, Burke K, Balu R, Amendolia O, et al. Cerebral Microdialysis. *Crit Care Nurs Clin North Am* (2016) 28(1):109–24. doi: 10.1016/j.cnc.2015.09.005
105. Hershey ND, Kennedy RT. In Vivo Calibration of Microdialysis Using Infusion of Stable-Isotope Labeled Neurotransmitters. *ACS Chem Neurosci* (2013) 4(5):729–36. doi: 10.1021/cn300199m
106. Baumeister FA, Rolinski B, Busch R, Emmrich P. Glucose Monitoring With Long-Term Subcutaneous Microdialysis in Neonates. *Pediatrics* (2001) 108 (5):1187–92. doi: 10.1542/peds.108.5.1187
107. Hage C, Mellbin L, Rydén L, Wernerman J. Glucose Monitoring by Means of an Intravenous Microdialysis Catheter Technique. *Diabetes Technol Ther* (2010) 12(4):291–5. doi: 10.1089/dia.2009.0150
108. Hack A, Busch V, Pascher B, Busch R, Bieger I, Gempel K, et al. Monitoring of Ketogenic Diet for Carnitine Metabolites by

Subcutaneous Microdialysis. *Pediatr Res* (2006) 60(1):93–6. doi: 10.1203/01.pdr.0000219479.95410.79

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REVIEW
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Congenital Hyperinsulinism: Current Laboratory-Based Approaches to the Genetic Diagnosis of a Heterogeneous Disease

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Congenital hyperinsulinism is characterised by the inappropriate release of insulin during hypoglycaemia. This potentially life-threatening disorder can occur in isolation, or present as a feature of syndromic disease. Establishing the underlying aetiology of the hyperinsulinism is critical for guiding medical management of this condition especially in children with diazoxide-unresponsive hyperinsulinism where the underlying genetics determines whether focal or diffuse pancreatic disease is present. Disease-causing single nucleotide variants affecting over 30 genes are known to cause persistent hyperinsulinism with mutations in the KATP channel genes (*ABCC8* and *KCNJ11*) most commonly identified in children with severe persistent disease. Defects in methylation, changes in chromosome number, and large deletions and duplications disrupting multiple genes are also well described in congenital hyperinsulinism, further highlighting the genetic heterogeneity of this condition. Next-generation sequencing has revolutionised the approach to genetic testing for congenital hyperinsulinism with targeted gene panels, exome, and genome sequencing being highly sensitive methods for the analysis of multiple disease genes in a single reaction. It should though be recognised that limitations remain with next-generation sequencing with no single application able to detect all reported forms of genetic variation. This is an important consideration for hyperinsulinism genetic testing as comprehensive screening may require multiple investigations.

Keywords: hyperinsulinism, hypoglycaemia, genetic screening, genetics, next generation sequencing - NGS

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INTRODUCTION

Persistent congenital hyperinsulinism (HI) is characterised by the inappropriate secretion of insulin during hypoglycaemia which continues beyond 3 months. A prompt diagnosis of HI and effective management of glucose levels is critical to prevent adverse outcomes (1).

Persistent HI affects approximately 1 in 13,500 to 1 in 45,000 new-borns in non-consanguineous populations (2–5). In some isolated communities where founder mutations have been reported, and in populations with high rates of consanguinity, the incidence can increase to approximately 1 in 3,000 (6, 7). At least 36 different genetic causes of HI have been reported which follow recessive, dominant, X-linked, or sporadic inheritance (**Table 1**). The underlying genetic aetiology will

determine whether the HI presents as isolated pancreatic disease or occurs as part of a rare syndrome.

Many laboratories provide genetic testing for congenital HI; however, strategies vary between testing centres both in terms of the genes that are screened and the types of variation that can be detected (23–25). The different approaches to testing employed by each laboratory could help explain the differences in the percentage of mutation positive cases between cohorts which range from 45% to 79% (3, 4, 26, 27). Furthermore, the large number of genes which cause HI, the variable penetrance observed both within and between families with the same disease-causing variants, and the multiple modes of inheritance reported can hinder genetic interpretation which will also impact on the pick-up rates reported by each laboratory.

In this review, we describe the genetic causes of HI and discuss the benefits and limitations of the different methodological approaches currently used for genetic screening of this condition.

GENETIC TYPES OF CONGENITAL HYPERINSULINISM

Disease-causing variants in 10 genes have been reported to cause isolated, persistent HI (**Table 1**). Loss-of-function variants in the *ABCC8* and *KCNJ11* genes, which encode the two subunits of the pancreatic beta-cell ATP-sensitive potassium (KATP) channel, are most common and reported in 30-66% of cases referred for genetic testing (3, 4, 26, 27). A wide range of clinical severity is associated with KATP-HI with the functionally mildest variants causing transient disease which responds well to diazoxide treatment (the frontline drug for HI), whilst the most functionally severe variants cause diazoxide-unresponsive HI that persists throughout childhood (8, 28, 29). For individuals with diazoxide-unresponsive HI, pancreatic resection may be required to prevent life-threatening hypoglycaemia. For these infants, rapid genetic testing of the KATP channel genes is critical as it will determine the histological subtype of disease. Identifying biallelic (two disease-causing variants on opposite alleles) or a single dominant KATP channel disease-causing variant confirms diffuse pancreatic disease. In contrast finding a paternally inherited, recessive KATP channel variant, predicts focal disease with a sensitivity of 97% (27, 30). In these individuals the variant is rendered homozygous by a second somatic genetic event within the pancreas (uniparental isodisomy) (31, 32). This can be genetically confirmed by testing the pancreatic tissue following a lesionectomy, which proves curative in most cases.

Clinical characteristics can help to predict some genetic forms of isolated HI. For example, high ammonia concentrations are a consistent feature of *GLUD1*-HI (12), a family history of Maturity-Onset Diabetes of the Young (MODY) can predict *HNF4A* or *HNF1A*- HI (16, 17), and exercise-induced HI suggests a role for the beta-cell disallowed gene, *SLC16A1* in disease pathogenesis (21).

Over 28 different syndromes which feature HI have been reported with the most common being Beckwith-Wiedemann syndrome (BWS) and Kabuki syndrome (33) (**Table 2**). The proportion of individuals with syndromic disease who present with HI varies between genetic subgroups. In some conditions HI is reported as a cardinal feature [e.g. Beckwith-Wiedemann syndrome (66)] whilst for others it is reported as a rare feature of the disease [e.g. Chromosome 9p deletions (40)]. Without genetic testing it can be hard to accurately diagnose syndromic disease, especially when HI is the presenting feature and dysmorphisms develop after birth, or when the clinical features are not specific to a genetic syndrome (67). For individuals with syndromic HI a genetic diagnosis is important as it will inform

TABLE 1 | Known genetic causes of isolated congenital hyperinsulinism and current approaches to genetic testing for this condition. A tick (✓) or cross (X) denote whether the form of genetic variation can be detected by the screening approach. None of the variants listed will be detected by methylation studies or array-CGH analysis. SNVs are single nucleotide variants, Indels are insertion/deletion variants and CNVs are copy number variants (deletions and duplications).

Gene	Zygosity	Mutation type	SangerSequencing[1]	Next Generation Sequencing			Ref
				Targeted Panel	Exome	Genome	
ABCC8	Dominant or recessive	SNVs/indels	✓	✓	✓[2]	✓	(8–10)
		Large CNVs	X	✓	✓	✓	
GCK	Dominant	SNVs/indels	✓	✓	✓	✓	(11)
GLUD1	Dominant	SNVs/indels	✓	✓	✓	✓	(12)
HADH	Recessive	SNVs/indels	✓	✓	✓[2]	✓	(13)
		Large CNVs	X	✓	✓	✓	(14)
HK1	Dominant	SNVs/indels	✓	✓	X	✓	(15)
		Large CNVs	X	✓	X	✓	
HNF1A	Dominant	SNVs/indels	✓	✓	✓	✓	(16)
HNF4A	Dominant	SNVs/indels	✓	✓	✓[2]	✓	(17)
		Large CNVs	X	✓	✓	✓	(18)
INSR	Dominant	SNVs/indels	✓	✓	✓	✓	(19)
KCNJ11	Dominant or recessive	SNVs/indels	✓	✓	✓	✓	(20)
SLC16A1	Dominant	SNVs/indels	✓	✓	X	✓	(21)

[1]*Sanger sequencing will not detect heterozygous deletions of duplications that extend beyond the targeted region. Homozygous deletions that encompass a primer binding site may be detected by a failure to amplify the sequence, but this will require verification by an independent method.*

[2]*Exome sequencing will not detect the deep intronic mutations or promoter mutations reported in these genes (22).*

TABLE 2 | Known genetic causes of syndromic disease in which congenital hyperinsulinism can be a rare or common feature and the current approaches to genetic testing for this condition. A tick (✓) or cross (X) denote whether the form of genetic variation can be detected by the screening approach. Methylation studies refer to methodologies that can detect changes in DNA methylation patterns (e.g. Epic array analysis, Methylation-specific MLPA). SNVs are single nucleotide variants, Indels are insertion/deletion variants and CNVs are copy number variants (deletions and duplications).

Gene	Zygosity	Syndrome	Mutation type	SangerSequencing[1]	Next Generation Sequencing			Array-CGH	Methylation studies	Ref
					TargetedPanel	Exome	Genome			
ABCC8	Recessive	Usher Syndrome	Large CNVs[2]	x	✓	✓	✓	X	X	(34)
ADK	Recessive	*ADK* deficiency	SNVs/indels	✓	✓	✓	✓	X	X	(35)
ALG3	Recessive	Congenital disorder of glycosylation	SNVs/indels	✓	✓	✓	✓	X	X	(36)
CACNA1D	Dominant	Primary aldosteronism, seizures & neurological abnormalities	SNVs/indels	✓	✓	✓	✓	X	X	(37)
CDKN1C	Dominant	Beckwith-Wiedemann	SNVs/indels	✓	✓	✓	✓	X	X	(38)
Chr5q35 deletion	Dominant	Sotos	Large CNVs	X	✓	✓	✓	✓	X	(39)
Chr9p deletion	Dominant	Chr9p deletion	Large CNVs	X	✓	✓	✓	✓	X	(40)
Chr11p15.5 loss of methylation	Dominant	Beckwith-Wiedemann	Imprinting abnormality	X	X[3]	X	X	X[3]	✓	(41)
CREBBP	Dominant	Rubinstein-Taybi	SNVs/indels	✓	✓	✓	✓	X	X	(42)
			Large CNVs	X	✓	✓	✓	X	X	
DIS3L2	Recessive	Perlman	SNVs/indels	✓	✓	✓	✓	X	X	(43, 44)
			Large CNVs	X	✓	✓	✓	X	X	
EIF2S3	X-linked recessive	MEHMO	SNVs/indels	✓	✓	✓	✓	X	X	(45)
EP300	Dominant	Rubinstein-Taybi	SNVs/indels	✓	✓	✓	✓	X	X	(42)
			Large CNVs	X	✓	✓	✓	X	X	
FAH	Recessive	Tyrosinaemia type I	SNVs/indels	✓	✓	✓	✓	X	X	(46)
FOXA2	Dominant	Syndromic	SNVs/indels	✓	✓	✓	✓	X	X	(47)
GPC3	X-linked recessive	Simpson-Golabi-Behmel	SNVs/indels	✓	✓	✓	✓	X	X	(48)
			Large CNVs	X	✓	✓	✓	X	X	
HNF4A	Dominant	Fanconi renotubular syndrome 4	SNV	✓	✓	✓	✓	X	X	(16)
HRAS	Dominant	Costello	SNVs/indels	✓	✓	✓	✓	X	X	(49)
KDM6A	X-linked dominant	Kabuki	SNVs/indels	✓	✓	✓	✓	X	X	(50)
			Large CNVs	X	✓	✓	✓	X	X	
KMT2D	Dominant	Kabuki	SNVs/indels	✓	✓	✓	✓	X	X	(51, 52)
			Large CNVs	X	✓	✓	✓	X	X	
MAGEL2	Dominant[4]	Schaaf-Yang	SNVs/indels	✓	✓	✓	✓	X	X	(53)
MPI	Recessive	Congenital disorder of glycosylation	SNVs/indels	✓	✓	✓	✓	X	X	(54)
NSD1	Dominant	Sotos	SNVs/indels	✓	✓	✓[5]	✓	X	X	(55–57)
			Large CNVs	X	✓	✓[5]	✓	X	X	
PHOX2B	Dominant	Congenital central hypoventilation	SNVs/indels	✓	✓	✓	✓	X	X	(58)
PMM2	Recessive	Polycystic Kidney Disease with HI	SNVs/indels	✓	✓	X	✓	X	X	(59)
		Congenital disorder of glycosylation	SNVs/indels	✓	✓	✓	✓	X	X	(60)
Trisomy 13	Dominant	Patau	Aneuploidy (Trisomy)	X	✓	✓	✓	✓	X	(61)
TRMT10A	Recessive	Syndromic	SNVs/indels	✓	✓	✓	✓	X	X	(62)
YARS	Recessive	Syndromic	SNVs/indels	✓	✓	✓	✓	X	X	(63)
45,X	Dominant	Turner	Aneuploidy (Monosomy)	X	✓	✓	✓	✓	X	(64)

on prognosis and allow for the effective monitoring of new features of the disease.

SANGER SEQUENCING

Causative genes for HI were historically screened by Sanger sequencing; an approach that allows a few hundred nucleotides (typically a single exon) to be rapidly sequenced in a single reaction. This is followed by semi-automated analysis by alignment and inspection of the DNA sequence. These constraints force laboratories to screen genes sequentially in descending order of prior probability based on clinical characteristics and how commonly disease-causing variants in the gene are identified. Whilst this phenotype-driven approach works well in many scenarios [for example in the rapid screening of KATP channel genes in individuals with diazoxide-unresponsive disease (68, 69)], the reliance of clinical features to guide testing can delay a genetic diagnosis for individuals with an atypical presentation. This is an important consideration for HI, as phenotypic variability is described within most genetic subgroups, for example the presence of normal ammonia levels in some children with *GLUD1*-HI (70, 71). Using the clinical characteristics to guide genetic testing in syndromic HI should also be applied with caution as additional features may develop after the diagnosis of HI (72).

A further major limitation of Sanger sequencing is its inability to detect heterozygous deletions and duplications that extend beyond the targeted region, changes in the number of chromosomes (aneuploidies), and defects in methylation, all of which have been reported to cause HI (**Table 1**).

Despite its limitations, Sanger sequencing remains a highly sensitive test for the rapid detection of single-nucleotide variants and small insertion/deletion variants (indels) in both the coding and non-coding regions of the genome. It can also detect mosaic variants (i.e. a genetic variant that is introduced during cell division that does not affect every cell within the body) that are present in the sampled tissue at a level of >8% (73). This is important, as disease-causing mosaic variants have been reported in the known HI genes including *KMT2D*, *KDM6A*, *NSD1*, and *CREBBP* (74–76).

NEXT-GENERATION SEQUENCING

Since 2005, next-generation sequencing has provided a method to allow for the simultaneous analysis of multiple genes in a single assay (77). This technology revolutionised diagnostic testing for genetically heterogeneous disorders such as HI by allowing for the parallel screening of all known disease-causing genes/genomic regions in a single assay at a much lower cost than Sanger sequencing. This led to a paradigm shift for conditions like syndromic HI where genetic testing can precede the development of the full clinical spectrum of disease, serving to make, rather than confirm, the clinical diagnosis (67).

[1]*Sanger sequencing will not detect heterozygous deletions of duplications that extend beyond the targeted region. Homozygous deletions that encompass a primer binding site may be detected by a failure to amplify the sequence, but this will require verification by an independent method.*
[2]*Congenital hyperinsulinism, profound congenital sensorineural deafness, enteropathy and renal tubular dysfunction is causes by a contiguous deletion extending over ABCC8 and USH1C.*
[3]*Rare deletions and duplications of the Chr11p15.5 imprinted region(s) can cause Beckwith Wiedemann syndrome (65). Their size and location will determine whether they can be detected by next-generation sequencing or microarray analysis.*
[4]*MAGEL2 is an imprinted gene, loss-of-function mutations only cause disease when present on the paternal allele.*
[5]*Intergenic mutations affecting NSD1 have been reported; these would not be detected by exome sequencing (55).*

Targeted Gene Panel Analysis by Next-Generation Sequencing

A targeted gene panel typically includes all known genetic causes of a disease and DNA samples are enriched for DNA in these loci prior to next-generation sequencing. For most targeted gene panels, the average coverage achieved often reaches many hundreds of reads over each base (78). This high-depth sequencing data can be exploited to detect changes in copy number over targeted regions and allows for the accurate detection of mosaic variants occurring at a level of >1% (73). Recent studies have shown that off-target reads generated during the sequencing process can be analysed to assess read-depth across the entire genome allowing for the detection of large deletions and duplications outside of targeted regions (79). These off-target reads have been used successfully to detect disease-causing deletions on chromosome 9p in individuals with HI (40). The potential to identify large deletions and duplications from off-target reads will though depend on the methodology used for the targeted next-generation sequencing; amplicon-based approaches that sequence PCR products will not generate the off-target sequencing data.

The major limitation of targeted next-generation sequencing is that it only allows screening of a predetermined list of genomic regions, and this list often differs between laboratories. For genetically heterogenous conditions such as HI, it is therefore important that clinicians who order panel testing are aware of which genes are included on the targeted panels and whether copy number analysis has been performed as this requires a separate bioinformatic analysis.

Exome and Genome Sequencing

The introduction of next-generation sequencing has enabled the rapid sequencing of the coding regions of all genes (the exome) or the entire human genome (coding and non-coding regions) at much lower cost than previous methods. The approach to the interpretation of exome and genome sequencing data will differ between centres with some analysing variants called within a pre-defined set of known disease-causing genes whilst other laboratories will perform a gene-agnostic analysis. The latter approach has the advantage of being able to identify new genes for HI, with recent successes including the discovery of the syndromic HI genes *CACNA1D*, *PMM2*, *FOXA2*, *TRMT10A*, *EIF2S3*, *YARS*, and *KMT2D* by exome sequencing and more recently the finding of regulatory variants deep within intron 2 of the beta-cell disallowed gene, *HK1*, by genome sequencing in individuals with isolated hyperinsulinism (15, 37, 45, 47, 51, 59, 62, 63). The ability of a laboratory to utilise next-generation sequencing data for genetic discovery will largely depend on their ability to perform robust genetic and functional studies to assess novel variation.

Exome sequencing targets the ~2% of the genome which codes for protein, making it a cheaper alternative to genome sequencing. This, together with the knowledge that 85% of known disease-causing mutations reside within coding regions, has led to exome sequencing being widely adopted within the clinical setting (80). For example, in the UK, rapid exome sequencing for acutely unwell neonates is available through the country's National Health Service with 38% of patients tested receiving a rapid diagnosis (81). Unlike targeted next-generation sequencing, which screens a predetermined list of genes, exome sequencing provides an extremely effective method to comprehensively analyse the coding regions and intron/exon boundaries of all known HI genes and to assess copy number status. The major limitation of the approach is that it will not detect non-coding mutations such as the deep intronic mutations reported in *ABCC8*, *HADH* and *HK1* or promoter variants in genes such as *HNF4A*, *PMM2*, and *SLC16A1* (15, 21, 22, 59, 82).

Genome sequencing represents the gold standard approach to genetic testing given its ability to detect the largest range of genetic variation. As well as providing data on coding and non-coding regions, genome sequencing can be used to search for structural changes, copy number variants (large deletions, duplications, and aneuploidies) and mosaic variants although the lower read depth achieved makes this a less sensitive approach for detecting low-level mosaic variants compared to targeted next-generation sequencing.

The costs associated with sequencing the entire genome and the large amount of data produced (approximately 200GB of processed data per sample versus 11GB per sample for exome sequencing) had prohibited the adoption of routine genome sequencing. Until recently it had been largely reserved for genetic screening when a disease-causing variant had not been detected by targeted next-generation sequencing or exome sequencing. This approach successful resulted in an increase in diagnostic yield for many rare genetic diseases (83, 84).

Improvements in sequencing capabilities leading to reduced costs are though now leading to the emergence of genome sequencing as a first line diagnostic test in specific healthcare settings, for example in the screening of some rare developmental disorders in the UK National Health Service (85). While genome sequencing is not the current approach for investigating the genetic cause of HI in many centres, it seems likely that this will become the first line test in the coming years.

NON SEQUENCING BASED METHODS TO DETECT COPY NUMBER VARIANTS AND METHYLATION DEFECTS

Aneuploidies and large deletions and duplications (copy number variants) are a rare but important cause of HI (**Tables 1** and **2**). Unlike Sanger sequencing, next-generation sequencing can detect these forms of genetic variation, but many laboratories will not routinely screen for them as a separate analysis pipeline is required. This is an important consideration when disease-causing variants are not detected in children with HI and particularly for those where there are additional syndromic features (**Table 2**).

Multiplex-ligation dependent probe amplification (MLPA) can detect disease-causing deletions and duplications in individuals with HI. This approach is commonly used to screen for deletions in the *ABCC8* gene and can detect

mosaicism (9). The usefulness of MLPA is limited by its ability to analyse a maximum of 60 different small genomic regions (generally single exons) in a single assay thus preventing the simultaneous analysis of all HI genes in which copy number changes have been reported.

Microarray-based comparative genomic hybridization (array CGH) is a well-established method that is used to detect large deletions/duplications and aneuploidies in individuals with HI. Unlike MLPA, array GCH is not able to detect low level mosaicism (<30% mosaicism for deletions and duplications and <10% for aneuploidies). The approach does however allow for the analysis of copy number variation across a greater percentage of the genome although the targeted region will vary across arrays and will not always target the regions known to cause HI with enough precision.

Current diagnostic sequencing approaches are also unable to detect changes in DNA methylation. Individuals with clinical suspicion of an imprinting disorder such as Beckwith-Wiedemann syndrome may therefore require additional methylation studies, such as methylation-specific MLPA (MS-MLPA) (86) or Infinium Methylation EPIC array analysis (87). Emerging technologies, such as Oxford Nanopore sequencing, may allow for the simultaneous detection of sequence variation and DNA methylation status but have not been widely used clinically. This technology does offer the hope of a single comprehensive test for genetically heterogeneous disorders like HI although to date it has mainly been used for genes that are hard to sequence by other methodologies (88–91).

FURTHER CONSIDERATIONS AND CONCLUDING REMARKS

Diagnostic testing for HI is routinely performed on DNA extracted from peripheral blood leukocytes, saliva, or buccal samples. For conditions such as HI it is important to consider the source of DNA being screened, given that somatic mutations which are only present in the pancreatic tissue have been reported (27, 92). Therefore, when a mutation is not identified in the blood, and a pancreatectomy has been performed, re-testing the known HI genes to search for a variant present only within the pancreatic DNA should be considered.

In conclusion, several different genetic approaches exist for routine diagnostic screening in HI with genome sequencing representing the gold standard approach to testing. For healthcare professionals managing this genetically heterogenous disorder it is important that the limitations of each approach including genome sequencing, are recognised as no single test can detect all known types of genetic variation reported in HI. This is particularly important when managing syndromic disease, where copy number variants or defects in methylation are common. Despite there being a broad range of genetic screening approaches that are available for HI, in reality the testing strategy is most likely to be influenced by the capabilities of the local genetic diagnostic laboratory, affordability and importantly how quickly the tests can be performed and results reported back. This is especially critical for children with diazoxide-unresponsive disease as identifying a paternally inherited KATP disease-causing variant suggests focal pancreatic disease which can be cured by lesionectomy.

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TH and SF performed the literature searches and reviews. TH, MJ, and SF drafted and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Helleskov A, Melikyan M, Globa E, Shcherderkina I, Poertner F, Larsen AM, et al. Both Low Blood Glucose and Insufficient Treatment Confer Risk of Neurodevelopmental Impairment in Congenital Hyperinsulinism: A Multinational Cohort Study. *Front Endocrinol (Lausanne)* (2017) 8:156. doi: 10.3389/fendo.2017.00156
2. Yau D, Laver TW, Dastamani A, Senniappan S, Houghton JAL, Shaikh G, et al. Using Referral Rates for Genetic Testing to Determine the Incidence of a Rare Disease: The Minimal Incidence of Congenital Hyperinsulinism in the UK is 1 in 28,389. *PloS One* (2020) 15(2):e0228417. doi: 10.1371/journal.pone.0228417
3. Rozenkova K, Malikova J, Nessa A, Dusatkova L, Bjorkhaug L, Obermannova B, et al. High Incidence of Heterozygous ABCC8 and HNF1A Mutations in Czech Patients With Congenital Hyperinsulinism. *J Clin Endocrinol Metab* (2015) 100(12):E1540–9. doi: 10.1210/jc.2015-2763
4. Mannisto JME, Jaaskelainen J, Otonkoski T, Huopio H. Long-Term Outcome and Treatment in Persistent and Transient Congenital Hyperinsulinism: A Finnish Population-Based Study. *J Clin Endocrinol Metab* (2021) 106(4): e1542–e51. doi: 10.1210/clinem/dgab024
5. Kawakita R, Sugimine H, Nagai S, Kawai M, Kusuda S, Yorifuji T. Clinical Characteristics of Congenital Hyperinsulinemic Hypoglycemia in Infant: A Nationwide Epidemiological Survey in Japan. *Nihon Shonika Gakkai Zasshi* (2011) 115:563–9.
6. Otonkoski T, Ammala C, Huopio H, Cote GJ, Chapman J, Cosgrove K, et al. A Point Mutation Inactivating the Sulfonylurea Receptor Causes the Severe Form of Persistent Hyperinsulinemic Hypoglycemia of Infancy in Finland. *Diabetes.* (1999) 48(2):408–15. doi: 10.2337/diabetes.48.2.408

7. Mathew PM, Young JM, Abu-Osba YK, Mulhern BD, Hammoudi S, Hamdan JA, et al. Persistent Neonatal Hyperinsulinism. *Clin Pediatr (Phila)* (1988) 27 (3):148–51. doi: 10.1177/000992288802700307
8. Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, et al. Mutations in the Sulfonylurea Receptor Gene in Familial Persistent Hyperinsulinemic Hypoglycemia of Infancy. *Science* (1995) 268(5209):426–9. doi: 10.1126/science.7716548
9. Flanagan S, Damhuis A, Banerjee I, Rokicki D, Jefferies C, Kapoor R, et al. Partial ABCC8 Gene Deletion Mutations Causing Diazoxide-Unresponsive Hyperinsulinaemic Hypoglycaemia. *Pediatr Diabetes* (2012) 13(3):285–9. doi: 10.1111/j.1399-5448.2011.00821.x
10. De Franco E, Saint-Martin C, Brusgaard K, Knight Johnson AE, Aguilar-Bryan L, Bowman P, et al. Update of Variants Identified in the Pancreatic Beta-Cell KATP Channel Genes KCNJ11 and ABCC8 in Individuals With Congenital Hyperinsulinism and Diabetes. *Hum Mutat* (2020) 41(5):884–905. doi: 10.1002/humu.23995
11. Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, et al. Familial Hyperinsulinism Caused by an Activating Glucokinase Mutation. *N Engl J Med* (1998) 338(4):226–30. doi: 10.1056/NEJM199801223380404
12. Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, et al. Hyperinsulinism and Hyperammonemia in Infants With Regulatory Mutations of the Glutamate Dehydrogenase Gene. *N Engl J Med* (1998) 338 (19):1352–7. doi: 10.1056/NEJM199805073381904
13. Clayton PT, Eaton S, Aynsley-Green A, Edginton M, Hussain K, Krywawych S, et al. Hyperinsulinism in Short-Chain L-3-Hydroxyacyl-CoA Dehydrogenase Deficiency Reveals the Importance of Beta-Oxidation in Insulin Secretion. *J Clin Invest* (2001) 108(3):457–65. doi: 10.1172/JCI200111294
14. Flanagan SE, Patch AM, Locke JM, Akcay T, Simsek E, Alaei M, et al. Genome-Wide Homozygosity Analysis Reveals HADH Mutations as a Common Cause of Diazoxide-Responsive Hyperinsulinemic-Hypoglycemia in Consanguineous Pedigrees. *J Clin Endocrinol Metab* (2011) 96(3):E498–502. doi: 10.1210/jc.2010-1906
15. Wakeling MN, Owens NDL, Hopkinson JR, Johnson MB, Houghton JAL, Dastamani A, et al. A Novel Disease Mechanism Leading to the Expression of a Disallowed Gene in the Pancreatic Beta-Cell Identified by non-Coding, Regulatory Mutations Controlling HK1. *medRxiv* (2021) 2021.12.03.21267240. doi: 10.1101/2021.12.03.21267240
16. Stanescu DE, Hughes N, Kaplan B, Stanley CA, De Leon DD. Novel Presentations of Congenital Hyperinsulinism Due to Mutations in the MODY Genes: HNF1A and HNF4A. *J Clin Endocrinol Metab* (2012) 97 (10):E2026–30. doi: 10.1210/jc.2012-1356
17. Pearson ER, Boj SF, Steele AM, Barrett T, Stals K, Shield JP, et al. Macrosomia and Hyperinsulinaemic Hypoglycaemia in Patients With Heterozygous Mutations in the HNF4A Gene. *PloS Med* (2007) 4(4):e118. doi: 10.1371/journal.pmed.0040118
18. Tung JY, Boodhansingh K, Stanley CA, De Leon DD. Clinical Heterogeneity of Hyperinsulinism Due to HNF1A and HNF4A Mutations. *Pediatr Diabetes* (2018) 19(5):910–6. doi: 10.1111/pedi.12655
19. Hojlund K, Hansen T, Lajer M, Henriksen JE, Levin K, Lindholm J, et al. A Novel Syndrome of Autosomal-Dominant Hyperinsulinemic Hypoglycemia Linked to a Mutation in the Human Insulin Receptor Gene. *Diabetes* (2004) 53(6):1592–8. doi: 10.2337/diabetes.53.6.1592
20. Thomas P, Ye Y, Lightner E. Mutation of the Pancreatic Islet Inward Rectifier Kir6.2 Also Leads to Familial Persistent Hyperinsulinemic Hypoglycemia of Infancy. *Hum Mol Genet* (1996) 5(11):1809–12. doi: 10.1093/hmg/5.11.1809
21. Otonkoski T, Jiao H, Kaminen-Ahola N, Tapia-Paez I, Ullah MS, Parton LE, et al. Physical Exercise-Induced Hypoglycemia Caused by Failed Silencing of Monocarboxylate Transporter 1 in Pancreatic Beta Cells. *Am J Hum Genet* (2007) 81(3):467–74. doi: 10.1086/520960
22. Flanagan SE, Xie W, Caswell R, Damhuis A, Vianey-Saban C, Akcay T, et al. Next-Generation Sequencing Reveals Deep Intronic Cryptic ABCC8 and HADH Splicing Founder Mutations Causing Hyperinsulinism by Pseudoexon Activation. *Am J Hum Genet* (2013) 92(1):131–6. doi: 10.1016/j.ajhg.2012.11.017
23. Novoa-Medina Y, Dominguez Garcia A, Quinteiro Gonzalez S, Garcia Cruz LM, Santana Rodriguez A. Congenital Hyperinsulinism in Gran Canaria, Canary Isles. *Pediatr (Engl Ed)* (2021) 95(2):93–100. doi: 10.1016/j.anpede.2020.08.006
24. Casertano A, Rossi A, Fecarotta S, Rosanio FM, Moracas C, Di Candia F, et al. An Overview of Hypoglycemia in Children Including a Comprehensive Practical Diagnostic Flowchart for Clinical Use. *Front Endocrinol (Lausanne)* (2021) 12:684011. doi: 10.3389/fendo.2021.684011
25. Razzaghy-Azar M, Saeedi S, Dayani SB, Enayati S, Abbasi F, Hashemian S, et al. Investigating Genetic Mutations in a Large Cohort of Iranian Patients With Congenital Hyperinsulinism. *J Clin Res Pediatr Endocrinol* (2022) 14 (1):87–95. doi: 10.4274/jcrpe.galenos.2021.2021.0071
26. Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K. Clinical and Molecular Characterisation of 300 Patients With Congenital Hyperinsulinism. *Eur J Endocrinol* (2013) 168(4):557–64. doi: 10.1530/EJE-12-0673
27. Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, et al. Genotype and Phenotype Correlations in 417 Children With Congenital Hyperinsulinism. *J Clin Endocrinol Metab* (2013) 98(2):E355–63. doi: 10.1210/jc.2012-2169
28. Taschenberger G, Mougey A, Shen S, Lester LB, LaFranchi S, Shyng SL. Identification of a Familial Hyperinsulinism-Causing Mutation in the Sulfonylurea Receptor 1 That Prevents Normal Trafficking and Function of KATP Channels. *J Biol Chem* (2002) 277(19):17139–46. doi: 10.1074/jbc.M200363200
29. Kumaran A, Kapoor RR, Flanagan SE, Ellard S, Hussain K. Congenital Hyperinsulinism Due to a Compound Heterozygous ABCC8 Mutation With Spontaneous Resolution at Eight Weeks. *Horm Res Paediatr* (2010) 73 (4):287–92. doi: 10.1159/000284394
30. Mohnike K, Wieland I, Barthlen W, Vogelgesang S, Empting S, Mohnike W, et al. Clinical and Genetic Evaluation of Patients With KATP Channel Mutations From the German Registry for Congenital Hyperinsulinism. *Horm Res Paediatr* (2014) 81(3):156–68. doi: 10.1159/000356905
31. de Lonlay P, Fournet JC, Rahier J, Gross-Morand MS, Poggi-Travert F, Foussier V, et al. Somatic Deletion of the Imprinted 11p15 Region in Sporadic Persistent Hyperinsulinemic Hypoglycemia of Infancy is Specific of Focal Adenomatous Hyperplasia and Endorses Partial Pancreatectomy. *J Clin Invest* (1997) 100(4):802–7. doi: 10.1172/JCI119594
32. Damaj L, le Lorch M, Verkarre V, Werl C, Hubert L, Nihoul-Fekete C, et al. Chromosome 11p15 Paternal Isodisomy in Focal Forms of Neonatal Hyperinsulinism. *J Clin Endocrinol Metab* (2008) 93(12):4941–7. doi: 10.1210/jc.2008-0673
33. Kostopoulou E, Dastamani A, Guemes M, Clement E, Caiulo S, Shanmugananda P, et al. Syndromic Forms of Hyperinsulinaemic Hypoglycaemia-A 15-Year Follow-Up Study. *Clin Endocrinol (Oxf)* (2021) 94(3):399–412. doi: 10.1111/cen.14393
34. Bitner-Glindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, et al. A Recessive Contiguous Gene Deletion Causing Infantile Hyperinsulinism, Enteropathy and Deafness Identifies the Usher Type 1C Gene. *Nat Genet* (2000) 26(1):56–60. doi: 10.1038/79178
35. Staufner C, Lindner M, Dionisi-Vici C, Freisinger P, Dobbelaere D, Douillard C, et al. Adenosine Kinase Deficiency: Expanding the Clinical Spectrum and Evaluating Therapeutic Options. *J Inherit Metab Dis* (2016) 39(2):273–83. doi: 10.1007/s10545-015-9904-y
36. Sun L, Eklund EA, Chung WK, Wang C, Cohen J, Freeze HH. Congenital Disorder of Glycosylation Id Presenting With Hyperinsulinemic Hypoglycemia and Islet Cell Hyperplasia. *J Clin Endocrinol Metab* (2005) 90(7):4371–5. doi: 10.1210/jc.2005-0250
37. Flanagan SE, Vairo F, Johnson MB, Caswell R, Laver TW, Lango Allen H, et al. A CACNA1D Mutation in a Patient With Persistent Hyperinsulinaemic Hypoglycaemia, Heart Defects, and Severe Hypotonia. *Pediatr Diabetes* (2017) 18(4):320–3. doi: 10.1111/pedi.12512
38. Hatada I, Ohashi H, Fukushima Y, Kaneko Y, Inoue M, Komoto Y, et al. An Imprinted Gene P57kip2 is Mutated in Beckwith-Wiedemann Syndrome. *Nat Genet* (1996) 14(2):171–3. doi: 10.1038/ng1096-171
39. Matsuo T, Ihara K, Ochiai M, Kinjo T, Yoshikawa Y, Kojima-Ishii K, et al. Hyperinsulinemic Hypoglycemia of Infancy in Sotos Syndrome. *Am J Med Genet A* (2013) 161A(1):34–7. doi: 10.1002/ajmg.a.35657
40. Banerjee I, Senniappan S, Laver TW, Caswell R, Zenker M, Mohnike K, et al. Refinement of the Critical Genomic Region for Congenital Hyperinsulinism

in the Chromosome 9p Deletion Syndrome. *Wellcome Open Res* (2019) 4:149. doi: 10.12688/wellcomeopenres.15465.1
41. Munns CF, Batch JA. Hyperinsulinism and Beckwith-Wiedemann Syndrome. *Arch Dis Child Fetal Neonatal Ed* (2001) 84(1):F67–9. doi: 10.1136/fn.84.1.F67
42. Welters A, El-Khairi R, Dastamani A, Bachmann N, Bergmann C, Gilbert C, et al. Persistent Hyperinsulinaemic Hypoglycaemia in Children With Rubinstein-Taybi Syndrome. *Eur J Endocrinol* (2019) 181(2):121–8. doi: 10.1530/EJE-19-0119
43. Henneveld HT, van Lingen RA, Hamel BC, Stolte-Dijkstra I, van Essen AJ. Perlman Syndrome: Four Additional Cases and Review. *Am J Med Genet* (1999) 86(5):439–46. doi: 10.1002/(SICI)1096-8628(19991029)86:5<439::AID-AJMG9>3.0.CO;2-4
44. Astuti D, Morris MR, Cooper WN, Staals RH, Wake NC, Fews GA, et al. Germline Mutations in DIS3L2 Cause the Perlman Syndrome of Overgrowth and Wilms Tumor Susceptibility. *Nat Genet* (2012) 44(3):277–84. doi: 10.1038/ng.1071
45. Gregory LC, Ferreira CB, Young-Baird SK, Williams HJ, Harakalova M, van Haaften G, et al. Impaired EIF2S3 Function Associated With a Novel Phenotype of X-Linked Hypopituitarism With Glucose Dysregulation. *EBioMedicine.* (2019) 42:470–80. doi: 10.1016/j.ebiom.2019.03.013
46. Baumann U, Preece MA, Green A, Kelly DA, McKiernan PJ. Hyperinsulinism in Tyrosinaemia Type I. *J Inherit Metab Dis* (2005) 28(2):131–5. doi: 10.1007/s10545-005-5517-1
47. Giri D, Vignola ML, Gualtieri A, Scagliotti V, McNamara P, Peak M, et al. Novel FOXA2 Mutation Causes Hyperinsulinism, Hypopituitarism With Craniofacial and Endoderm-Derived Organ Abnormalities. *Hum Mol Genet* (2017) 26(22):4315–26. doi: 10.1093/hmg/ddx318
48. Terespolsky D, Farrell SA, Siegel-Bartelt J, Weksberg R. Infantile Lethal Variant of Simpson-Golabi-Behmel Syndrome Associated With Hydrops Fetalis. *Am J Med Genet* (1995) 59(3):329–33. doi: 10.1002/ajmg.1320590310
49. Alexander S, Ramadan D, Alkhayyat H, Al-Sharkawi I, Backer KC, El-Sabban F, et al. Costello Syndrome and Hyperinsulinemic Hypoglycemia. *Am J Med Genet A* (2005) 139(3):227–30. doi: 10.1002/ajmg.a.31011
50. Gole H, Chuk R, Coman D. Persistent Hyperinsulinism in Kabuki Syndrome 2: Case Report and Literature Review. *Clin Pract* (2016) 6(3):848. doi: 10.4081/cp.2016.848
51. Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, et al. Exome Sequencing Identifies MLL2 Mutations as a Cause of Kabuki Syndrome. *Nat Genet* (2010) 42(9):790–3. doi: 10.1038/ng.646
52. White SM, Thompson EM, Kidd A, Savarirayan R, Turner A, Amor D, et al. Growth, Behavior, and Clinical Findings in 27 Patients With Kabuki (Niikawa-Kuroki) Syndrome. *Am J Med Genet A* (2004) 127A(2):118–27. doi: 10.1002/ajmg.a.20674
53. Soden SE, Saunders CJ, Willig LK, Farrow EG, Smith LD, Petrikin JE, et al. Effectiveness of Exome and Genome Sequencing Guided by Acuity of Illness for Diagnosis of Neurodevelopmental Disorders. *Sci Transl Med* (2014) 6(265):265ra168. doi: 10.1126/scitranslmed.3010076
54. Deeb A, Al Amoodi A. A Novel Homozygous Mutation in the Mannose Phosphate Isomerase Gene Causing Congenital Disorder of Glycation and Hyperinsulinemic Hypoglycemia in an Infant. *Clin Case Rep* (2018) 6(3):479–83. doi: 10.1002/ccr3.1387
55. Carrasco Salas P, Palma Milla C, Lezana Rosales JM, Benito C, Franco Freire S, Lopez Siles J. Hyperinsulinemic Hypoglycemia in a Patient With an Intragenic NSD1 Mutation. *Am J Med Genet A* (2016) 170A(2):544–6. doi: 10.1002/ajmg.a.37440
56. Grand K, Gonzalez-Gandolfi C, Ackermann AM, Aljeaid D, Bedoukian E, Bird LM, et al. Hyperinsulinemic Hypoglycemia in Seven Patients With *De Novo* NSD1 Mutations. *Am J Med Genet A* (2019) 179(4):542–51. doi: 10.1002/ajmg.a.61062
57. Douglas J, Tatton-Brown K, Coleman K, Guerrero S, Berg J, Cole TR, et al. Partial NSD1 Deletions Cause 5% of Sotos Syndrome and are Readily Identifiable by Multiplex Ligation Dependent Probe Amplification. *J Med Genet* (2005) 42(9):e56. doi: 10.1136/jmg.2005.031930
58. Hennewig U, Hadzik B, Vogel M, Meissner T, Goecke T, Peters H, et al. Congenital Central Hypoventilation Syndrome With Hyperinsulinism in a Preterm Infant. *J Hum Genet* (2008) 53(6):573–7. doi: 10.1007/s10038-008-0275-1
59. Cabezas OR, Flanagan SE, Stanescu H, Garcia-Martinez E, Caswell R, Lango-Allen H, et al. Polycystic Kidney Disease With Hyperinsulinemic Hypoglycemia Caused by a Promoter Mutation in Phosphomannomutase 2. *J Am Soc Nephrol* (2017) 28(8):2529–39. doi: 10.1681/ASN.2016121312
60. Bohles H, Sewell AA, Gebhardt B, Reinecke-Luthge A, Kloppel G, Marquardt T. Hyperinsulinaemic Hypoglycaemia–Leading Symptom in a Patient With Congenital Disorder of Glycosylation Ia (Phosphomannomutase Deficiency). *J Inherit Metab Dis* (2001) 24(8):858–62. doi: 10.1023/A:1013944308881
61. Tamame T, Hori N, Homma H, Yoshida R, Inokuchi M, Kosaki K, et al. Hyperinsulinemic Hypoglycemia in a Newborn Infant With Trisomy 13. *Am J Med Genet A* (2004) 129A(3):321–2. doi: 10.1002/ajmg.a.30147
62. Gillis D, Krishnamohan A, Yaacov B, Shaag A, Jackman JE, Elpeleg O. TRMT10A Dysfunction is Associated With Abnormalities in Glucose Homeostasis, Short Stature and Microcephaly. *J Med Genet* (2014) 51(9):581–6. doi: 10.1136/jmedgenet-2014-102282
63. Zeiad R, Ferren EC, Young DD, De Lancy SJ, Dedousis D, Schillaci LA, et al. A Novel Homozygous Missense Mutation in the YARS Gene: Expanding the Phenotype of YARS Multisystem Disease. *J Endocr Soc* (2021) 5(2):bvaa196. doi: 10.1210/jendso/bvaa196
64. Alkhayyat H, Christesen HB, Steer J, Stewart H, Brusgaard K, Hussain K. Mosaic Turner Syndrome and Hyperinsulinaemic Hypoglycaemia. *J Pediatr Endocrinol Metab* (2006) 19(12):1451–7. doi: 10.1515/JPEM.2006.19.12.1451
65. Brioude F, Lacoste A, Netchine I, Vazquez MP, Auber F, Audry G, et al. Beckwith-Wiedemann Syndrome: Growth Pattern and Tumor Risk According to Molecular Mechanism, and Guidelines for Tumor Surveillance. *Horm Res Paediatr* (2013) 80(6):457–65. doi: 10.1159/000355544
66. Brioude F, Kalish JM, Mussa A, Foster AC, Bliek J, Ferrero GB, et al. Expert Consensus Document: Clinical and Molecular Diagnosis, Screening and Management of Beckwith-Wiedemann Syndrome: An International Consensus Statement. *Nat Rev Endocrinol* (2018) 14(4):229–49. doi: 10.1038/nrendo.2017.166
67. Hoermann H, El-Rifai O, Schebek M, Lodefalk M, Brusgaard K, Bachmann N, et al. Comparative Meta-Analysis of Kabuki Syndrome With and Without Hyperinsulinaemic Hypoglycaemia. *Clin Endocrinol (Oxf)* (2020) 93(3):346–54. doi: 10.1111/cen.14267
68. Hewat TI, Yau D, Jerome JCS, Laver TW, Houghton JAL, Shields BM, et al. Birth Weight and Diazoxide Unresponsiveness Strongly Predict the Likelihood of Congenital Hyperinsulinism Due to a Mutation in ABCC8 or KCNJ11. *Eur J Endocrinol* (2021) 185(6):813–8. doi: 10.1530/EJE-21-0476
69. Banerjee I, Skae M, Flanagan SE, Rigby L, Patel L, Didi M, et al. The Contribution of Rapid KATP Channel Gene Mutation Analysis to the Clinical Management of Children With Congenital Hyperinsulinism. *Eur J Endocrinol* (2011) 164(5):733–40. doi: 10.1530/EJE-10-1136
70. Brandt A, Agarwal N, Giri D, Yung Z, Didi M, Senniappan S. Hyperinsulinism Hyperammonaemia (HI/HA) Syndrome Due to GLUD1 Mutation: Phenotypic Variations Ranging From Late Presentation to Spontaneous Resolution. *J Pediatr Endocrinol Metab* (2020) 33(5):675–9. doi: 10.1515/jpem-2019-0416
71. Kapoor RR, Flanagan SE, Fulton P, Chakrapani A, Chadefaux B, Ben-Omran T, et al. Hyperinsulinism-Hyperammonaemia Syndrome: Novel Mutations in the GLUD1 Gene and Genotype-Phenotype Correlations. *Eur J Endocrinol* (2009) 161(5):731–5. doi: 10.1530/EJE-09-0615
72. Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml J, Nelakuditi V, et al. Congenital Hyperinsulinism as the Presenting Feature of Kabuki Syndrome: Clinical and Molecular Characterization of 9 Affected Individuals. *Genet Med* (2019) 21(1):233–42. doi: 10.1038/s41436-018-0013-9
73. Brewer CJ, Gillespie M, Fierro J, Scaringe WA, Li JM, Lee CY, et al. The Value of Parental Testing by Next-Generation Sequencing Includes the Detection of Germline Mosaicism. *J Mol Diagn* (2020) 22(5):670–8. doi: 10.1016/j.jmoldx.2020.02.001
74. Murakami H, Tsurusaki Y, Enomoto K, Kuroda Y, Yokoi T, Furuya N, et al. Update of the Genotype and Phenotype of KMT2D and KDM6A by Genetic Screening of 100 Patients With Clinically Suspected Kabuki Syndrome. *Am J Med Genet A* (2020) 182(10):2333–44. doi: 10.1002/ajmg.a.61793
75. Castronovo C, Rusconi D, Crippa M, Giardino D, Gervasini C, Milani D, et al. A Novel Mosaic NSD1 Intragenic Deletion in a Patient With an Atypical

Phenotype. *Am J Med Genet A* (2013) 161A(3):611–8. doi: 10.1002/ajmg.a.35814
76. Lin S, He Z, Huang L, Liu J, Lei T, Wu J, et al. Case Report: Low-Level Maternal Mosaicism of a Novel CREBBP Variant Causes Recurrent Rubinstein-Taybi Syndrome in Two Siblings of a Chinese Family. *Front Genet* (2021) 12:640992. doi: 10.3389/fgene.2021.640992
77. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, et al. Genome Sequencing in Microfabricated High-Density Picolitre Reactors. *Nature.* (2005) 437(7057):376–80. doi: 10.1038/nature03959
78. Cleveland MH, Zook JM, Salit M, Vallone PM. Determining Performance Metrics for Targeted Next-Generation Sequencing Panels Using Reference Materials. *J Mol Diagn* (2018) 20(5):583–90. doi: 10.1016/j.jmoldx.2018.04.005
79. Laver TW, De Franco E, Johnson MB, Patel KA, Ellard S, Weedon MN, et al. SavvyCNV: Genome-Wide CNV Calling From Off-Target Reads. *PloS Comput Biol* (2022) 18(3):e1009940. doi: 10.1371/journal.pcbi.1009940
80. Scacheri CA, Scacheri PC. Mutations in the Noncoding Genome. *Curr Opin Pediatr* (2015) 27(6):659–64. doi: 10.1097/MOP.0000000000000283
81. McDermott H, Baple E, Ellard S, Naik S. 1435 Rapid Exome Sequencing in Acutely Unwell Children – Providing New Diagnostic Options in Intensive Care Settings. *Arch Dis Child* (2021), :106(Suppl 1):A1–A514. doi: 10.1136/archdischild-2021-rcpch.641
82. Colclough K, Bellanne-Chantelot C, Saint-Martin C, Flanagan SE, Ellard S. Mutations in the Genes Encoding the Transcription Factors Hepatocyte Nuclear Factor 1 Alpha and 4 Alpha in Maturity-Onset Diabetes of the Young and Hyperinsulinemic Hypoglycemia. *Hum Mutat* (2013) 34(5):669–85. doi: 10.1002/humu.22279
83. Mattick JS, Dinger M, Schonrock N, Cowley M. Whole Genome Sequencing Provides Better Diagnostic Yield and Future Value Than Whole Exome Sequencing. *Med J Aust* (2018) 209(5):197–9. doi: 10.5694/mja17.01176
84. Investigators GPP, Smedley D, Smith KR, Martin A, Thomas EA, McDonagh EM, et al. 100,000 Genomes Pilot on Rare-Disease Diagnosis in Health Care - Preliminary Report. *N Engl J Med* (2021) 385(20):1868–80. doi: 10.1056/NEJMoa2035790
85. Caulfield M, Davies J, Dennys M, Elbahy L, Fowler T, Hill S, et al. The National Genomics Research and Healthcare Knowledgebase. (2020).
86. Scott RH, Douglas J, Baskcomb L, Nygren AO, Birch JM, Cole TR, et al. Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA) Robustly Detects and Distinguishes 11p15 Abnormalities Associated With Overgrowth and Growth Retardation. *J Med Genet* (2008) 45(2):106–13. doi: 10.1136/jmg.2007.053207
87. Aref-Eshghi E, Kerkhof J, Pedro VP, France GD, Barat-Houari M, Ruiz-Pallares N, et al. Evaluation of DNA Methylation Episignatures for Diagnosis and Phenotype Correlations in 42 Mendelian Neurodevelopmental Disorders. *Am J Hum Genet* (2021) 108(6):1161–3. doi: 10.1016/j.ajhg.2021.04.022
88. Nowak A, Murik O, Mann T, Zeevi DA, Altarescu G. Detection of Single Nucleotide and Copy Number Variants in the Fabry Disease-Associated GLA Gene Using Nanopore Sequencing. *Sci Rep* (2021) 11(1):22372. doi: 10.1038/s41598-021-01749-7
89. Leija-Salazar M, Sedlazeck FJ, Toffoli M, Mullin S, Mokretar K, Athanasopoulou M, et al. Evaluation of the Detection of GBA Missense Mutations and Other Variants Using the Oxford Nanopore MinION. *Mol Genet Genomic Med* (2019) 7(3):e564. doi: 10.1002/mgg3.564
90. Mahaweni NM, Olieslagers TI, Rivas IO, Molenbroeck SJJ, Groeneweg M, Bos GMJ, et al. A Comprehensive Overview of FCGR3A Gene Variability by Full-Length Gene Sequencing Including the Identification of V158F Polymorphism. *Sci Rep* (2018) 8(1):15983. doi: 10.1038/s41598-018-34258-1
91. Minervini CF, Cumbo C, Orsini P, Brunetti C, Anelli L, Zagaria A, et al. TP53 Gene Mutation Analysis in Chronic Lymphocytic Leukemia by Nanopore MinION Sequencing. *Diagn Pathol* (2016) 11(1):96. doi: 10.1186/s13000-016-0550-y
92. Houghton JA, Banerjee I, Shaikh G, Jabbar S, Laver TW, Cheesman E, et al. Unravelling the Genetic Causes of Mosaic Islet Morphology in Congenital Hyperinsulinism. *J Pathol Clin Res* (2020) 6(1):12–6. doi: 10.1002/cjp2.144

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Families' Experiences of Continuous Glucose Monitoring in the Management of Congenital Hyperinsulinism: A Thematic Analysis

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Background and Aims: In patients with congenital hyperinsulinism (CHI), recurrent hypoglycaemia can lead to longstanding neurological impairments. At present, glycaemic monitoring is with intermittent fingerprick blood glucose testing but this lacks utility to identify patterns and misses hypoglycaemic episodes between tests. Although continuous glucose monitoring (CGM) is well established in type 1 diabetes, its use has only been described in small studies in patients with CHI. In such studies, medical perspectives have been provided without fully considering the views of families using CGM. In this qualitative study, we aimed to explore families' experiences of using CGM in order to inform future clinical strategies for the management of CHI.

Methods: Ten patients with CHI in a specialist centre used CGM for twelve weeks. All were invited to participate. Semi-structured interviews were conducted with nine families in whom patient ages ranged between two and seventeen years. Transcripts of the audio-recorded interviews were analysed using an inductive thematic analysis method.

Results: Analysis revealed five core themes: CGM's function as an educational tool; behavioural changes; positive experiences; negative experiences; and design improvements. Close monitoring and retrospective analysis of glucose trends allowed for enhanced understanding of factors that influenced glucose levels at various times of the day. Parents noted more hypoglycaemic episodes than previously encountered through fingerprick tests; this new knowledge prompted modification of daily routines to prevent and improve the management of hypoglycaemia. CGM use was viewed favourably as offering parental reassurance, reduced fingerprick tests and predictive warnings. However, families also reported unfavourable aspects of alarms and questionable accuracy at low glucose levels. Adolescents were frustrated by the short proximity range for data transmission resulting in the need to always carry a separate

receiver. Overall, families were positive about the use of CGM but expected application to be tailored to their child's medical condition.

Conclusions: Patients and families with CHI using CGM noticed trends in glucose levels which motivated behavioural changes to reduce hypoglycaemia with advantages outweighing disadvantages. They expected CHI-specific modifications to enhance utility. Future design of CGM should incorporate end users' opinions and experiences for optimal glycaemic monitoring of CHI.

Keywords: congenital hyperinsulinism, continuous glucose monitoring, thematic analysis, interviews, experiences

INTRODUCTION

Congenital hyperinsulinism (CHI) is a disorder characterised by severe hypoglycaemia due to inappropriate secretion of insulin by the pancreatic β-cells (1). Despite CHI being a rare disorder with an estimated incidence of 1:28,389 in the UK, it is the most common cause of persistent hypoglycaemia in children (2, 3).

In addition to causing hypoglycaemia, excessive and dysregulated insulin secretion suppresses the production of ketones, which normally act as an important alternative fuel to preserve neuronal function when there is insufficient glucose (4). CHI is therefore well-recognised for its association with poor neurodevelopmental outcomes in patients, with 15% - 48% of children with CHI having long-term neurodevelopmental impairment at follow-up (4–7). Prompt detection and treatment of hypoglycaemia in CHI is therefore vital. Standard clinical practice for the monitoring of glucose in CHI is with regular fingerprick blood glucose testing using a point-of-care device or a home glucometer, whilst management includes the optimisation of feeds, medications such as diazoxide and octreotide, and pancreatectomy dependent on the type of CHI (8).

Advancements in technology have resulted in the increasingly widespread use of continuous glucose monitoring (CGM) rather than fingerprick blood glucose monitoring in patients with type 1 diabetes mellitus (9). The minimally invasive CGM device is attached to the skin, detects changes in interstitial glucose levels and displays the readings to the user every five minutes via a hand-held receiver or a mobile phone (10). Frequent glucose monitoring is a cornerstone of intensive CHI management and CGM provides an attractive alternative to intermittent fingerprick testing, which has too low a granularity to offer trend information and can miss hypoglycaemic episodes in between measurements.

Although there is heightened interest about the use of CGM in patients with CHI, the clinical utility has not been explored carefully (11). There is growing interest in the application of CGM to improve glycaemic control in neonates with CHI (12, 13). Win et al. reported that CGM showed rapid fluctuations in glucose levels in fourteen neonates with CHI alongside persistent hypoglycaemia, reflecting the high risk of undetected hypoglycaemic episodes when managed on intermittent fingerprick glucose tests (14). Rayannavar *et al*'s observational study demonstrated a high false positive rate for hypoglycaemia readings for children with CHI over a two-week period; the authors determined that CGM should be used as an adjunct to glucose monitoring rather than a sole monitoring device due to its suboptimal accuracy (15). More recently, Worth et al. conducted an exploratory study in which CGM was used to collect detailed glycaemic data over a period of four to ten days in twenty-three patients with CHI and found that there was an increased risk of hypoglycaemia in the early hours of the morning (16).

The HI Global Registry, a patient-powered CHI registry, found that 49% of parents of children under five reported the management of CHI to be 'demanding' (17). A recent review considering the unmet needs of patients and families with CHI suggested the wider application of CGM, while recognising shortcomings in its present use (11). While CGM has the potential to improve glycaemic monitoring and hence outcomes in CHI, it is vital that end users' opinions on using the device are gathered before broader implementation. By way of a questionnaire, Vijayanand et al. sought to evaluate parents' experiences of CGM; the majority preferred using CGM to a fingerprick glucometer, although seven out of the eleven parents felt that it was not accurate all the time (18). However, deeper analysis of patients' and families' experiences through interview was not available. In our study, we aimed to gain a richer understanding of the experiences of families using CGM; we conducted the first qualitative study employing thematic analysis of semi-structured interviews with adolescents with CHI and parents of young children with CHI.

METHODS

We undertook a qualitative study to perform an in-depth analysis of families' experiences of CGM use in a small group (n=9) of CHI patients. As little is known about the experiences of CGM in families with CHI, a rare disease of hypoglycaemia, qualitative methods are ideal for investigating the subject in a small targeted population in contrast to structured questionnaires in a larger group (19). They allow for participants to freely disclose their thoughts and experiences without constraint, providing a unique depth of understanding that cannot be gained from a closed question survey (20). Futhermore, qualitative methods and analysis enable open

representation of user perceived concepts and themes reducing prior prejudice and investigator bias from influencing the results.

This qualitative study was the second phase of a related study in which patients with CHI had used CGM (Dexcom G6) for twelve weeks and received expert review of glucose profiles at weeks eight and twelve without pharmacological intervention (21). The Dexcom G6 used in the study employed a separate hand-held receiver. CGM glucose was reported in mmol/L; as per UK consensus, hypoglycaemia was defined as less than 3.5mmol/l (63mg/dL) (2). If CGM reported a glucose level of less than 3.5 mmol/l, families were instructed to also check the glucose level with a fingerprick blood glucose test and treat hypoglycaemia if confirmed.

During the first four weeks, families were blinded to the glucose readings, which are usually displayed in real time by CGM. They were then able to use CGM unblinded, with readings available for four weeks, before a review of the glucose trends during this time period was conducted with a research clinician. For the final four weeks of the study, the device was blinded once more and followed by a final review of glucose profile. For inclusion in the study, patients with CHI were approached through the Northern Congenital Hyperinsulinism Service (NORCHI), Manchester, United Kingdom. Patients were eligible for inclusion to the CGM study if they were under the age of eighteen years and receiving medication for treatment of confirmed CHI.

For this qualitative study, inclusion criteria included parents/guardians of children with CHI, adolescents with CHI (defined as greater than twelve years of age) and the use of CGM for at least six weeks during the study period (including four weeks of unblinded CGM). All families of participants of the initial study were approached to be included in the qualitative phase of the study. All ten families initially consented to participate in the study. However, one family did not maintain contact thus preventing them from inclusion in the interviews. Two of the five adolescents did not participate in the interview alongside their parent(s) after having previously consented to participation; this was due to fatigue at the time of interview for Patient 3; and the mother of Patient 5 only being available for interview during the day, whilst her daughter was at school.

The protocol, consent forms and interview topic guide were approved by the ethics committee of the University of Manchester and the Health Research Authority of the National Health Service (REC reference 07/H1010/88). Adolescents and parents of the younger children gave written informed consent. Further verbal consent was obtained at routine research follow-up clinic appointments prior to organising interviews. Incentives were not provided for participation.

Semi-structured interviews with parents and adolescents were conducted in December 2021 via videoconferencing platforms to explore families' experiences of CGM use. At the time of interview, all families had used CGM for twelve weeks during the study and had continued to use unblinded CGM for a further four weeks. The semi-structured approach was selected as it permitted the flexibility for participants to speak about the issues they perceived to be most important, and for those to be explored, whilst the topic guide helped to ensure data collection remained relevant to the study aim. The **The Appendix within the Supplementary Material** includes the interview topic guide which consisted of prompts and questions on families' opinions and experiences of CGM and the perceived benefits and challenges of using CGM. The interview guide was developed through consensus with researchers, clinicians and psychologists with expertise in CHI.

Braun and Clarke's approach to thematic analysis was chosen as the mode of analysis as it allowed a pragmatic approach to analyse participants' lived experiences, behaviours, and perspectives (22). Its flexibility also allows for use on small datasets, which is especially important given CHI is a rare condition. Thematic analysis was favoured over interpretative phenomenological analysis, which can also be conducted on small homogenous samples, as it places greater emphasis on patterns across participants whilst the latter phenomenological approach notes patterns but focuses on how each unique individual makes sense of events (23).

In terms of reflexivity, interviews were conducted by a clinical research paediatrician who was not involved in the first phase of the CGM research study and did not have prior information about the patients or families. Importantly, the families understood that reporting on their experiences would not affect their potential future supplies of CGM equipment or clinical care. Members of the same family were interviewed together and each interview lasted between twenty and thirty minutes. Respondent validation, whereby participants confirmed accuracy of the information they had provided, was conducted throughout the interview process.

Interviews were transcribed verbatim and subsequently checked for accuracy against the audio recordings. This stage, along with repeated reading of the transcripts and noting early impressions, allowed for further familiarisation with the data. Personal identifiers were removed in the transcription process.

As there was little predetermined knowledge about CGM experiences in CHI, a predominantly inductive approach was used to code the data. Hence, research findings were derived from the data rather than using a pre-defined coding framework. It was not deemed appropriate to use multiple coders in this thematic analysis approach; inter-coder reliability merely shows that researchers have been trained to interpret data in similar ways (24, 25). Qualitative data analysis software (NVivo) was used to facilitate the application of initial codes to the entire dataset. Multiple codes were then combined to create themes, which captured common, recurring patterns across the data that described and explained participants' experiences. Prior to defining and naming of the themes, they were refined by reviewing all collated extracts for each theme to ensure there was sufficient supporting data.

RESULTS

Nine families were included in the study of which there were five parents of younger children, five parents of adolescents and three

adolescents. For each patient with CHI, at least one parent or patient was involved in the interview as described in **Table 1**. The demographics of patients, alongside CHI medications and the time since diagnosis are also presented in **Table 1**.

The results are presented as five major themes that were derived from the data:

1. Positive Experiences
2. Educational Tool
3. Behavioural Change
4. Negative Experiences
5. Design Improvements.

A rich and detailed analysis of the themes are accompanied by illustrative quotations to ensure robustness, whilst **Table 2** provides a summary of the themes in families' experiences of CGM in CHI.

Positive Experiences

Whilst families had become accustomed to living with CHI, their day-to-day life was felt to be less stressful with CGM as they were reassured about normoglycaemia, especially at night-time. *"But again, it's just peace of mind for parents that - just to see what's happening. Especially at night - if it works at night when she's poorly then I don't have to prick her as it might wake her up. So it's really good at night." – P9.*

Managing the condition was also perceived to be simpler with CGM enabling families to have more time to focus on other matters. Older siblings were also able to gain a sense of responsibility by helping out. *"When you've got a lot of other medical issues going on, it's just one thing that makes life a lot easier. Life's quite hectic. So we've got that one little thing that you ain't got to do which is like checking his blood sugars every time we eat."- P11. "Even our teenage children - they can be aware of it as well, because they haven't got to start messing with the pins and whatever. They can just look on the monitor. Gives us a little bit, not a lot, but a bit more leeway of doing other things in the house." – P10.*

A significant positive outcome through the use of CGM was the reduced number of fingerprick tests required: *"Also I like the fact I'm not having to check his bloods myself as much so that saves his little fingers."- P5.* Parents of younger children felt that day-to-day life for their children was less disrupted, especially at nursery and when playing outside, which would often require finding a space to remove clothing to do fingerprick tests. One parent [P6] discussed the environment: they perceived CGM to be more environmentally friendly than fingerprick monitoring techniques, which resulted in a perceived increase of non-recyclable wastage of testing strips and needles.

Parents of adolescents liked that CGM assisted them with objective evidence of hypoglycaemic episodes that could not be ignored continually by their children. Previously, as they were asymptomatic, the adolescent would often report that a fingerprick test was not required. *"We were saying to him, well I know for a fact that your sugars are 2.5, you need to have something to eat" – P4 "But now we both can see that, okay, it's low, and she'll read it's low, rather than us arguing." – P3.*

TABLE 1 | Interview participants and demographics of patients with CHI.

Interview Participant	Patient	Age at Time of Interview/years	Gender	Time since diagnosis of CHI/years	Genetics	Medications
Participant 1 [P1] -Father of Patient 1	Patient 1	3.1	Male	3.1	Homozygous ABCC8 mutation	Subcutaneous injections of octreotide three times daily
Participant 2 [P2] -Patient 2	Patient 2	14.5	Female	14.5	Paternally inherited KCNJ11 mutation	Oral diazoxide twice daily
Participant 3 [P3] -Mother of Patient 2						
Participant 4 [P4] -Mother of Patient 3	Patient 3	12.3	Male	11.9	No genetic cause identified	Oral diazoxide twice daily
Participant 5 [P5] -Mother of Patient 4	Patient 4	5.4	Male	5.4	Maternally inherited ABCC8 mutation	Oral diazoxide three times daily
Participant 6 [P6] -Mother of Patient 5	Patient 5	13.3	Female	13.0	HADH mutation	Oral diazoxide twice daily
Participant 7 [P7] -Patient 6	Patient 6	17.7	Male	7.4	GCK mutation	Oral diazoxide twice daily
Participant 8 [P8] -Mother of Patient 6						
Participant 9 [P9] -Mother of Patient 7	Patient 7	3.2	Female	3.0	No genetic cause identified	Oral diazoxide three times daily, chlorothiazide twice daily, cornstarch
Participant 10 [P10] -Mother of Patient 8	Patient 8	2.1	Male	2.1	HNF4A mutation	Oral diazoxide three times daily
Participant 11 [P11] -Father of Patient 8						
Participant 12 [P12] -Patient 9	Patient 9	17.3	Male	17.1	GLUD1 mutation	Oral diazoxide three times daily
Participant 13 [P13] -Mother of Patient 9						

TABLE 2 | Description of 5 major themes and subthemes in families' experiences of CGM use in CHI.

Theme	Positive Experiences	Educational Tool	Behavioural Change	Negative Experiences	Design Improvements
Theme Description	Factors regarded as positive/helpful by participants	Learning from CGM to improve management	Changes to routine due to CGM	Factors regarded as negative by participants	Refinements to design of CGM
Subthemes	Reassurance	New knowledge of glucose trends	Timing of meals changed	Alarms	Increase receiver range
	Less stressful management	More hypoglycaemia than previously thought	Ensured medications given on time	The need to carry receiver due to range	Incorporate wearable receiver
	Reduced fingerprick tests	Heightened awareness of hypoglycaemic times of the day	Improved family dynamics	Accuracy	Sensor size
	Glucose trend predictions	Reflection on reasons for hypoglycaemias	Adolescents taking increased responsibility for own condition	Sensor insertion	Tailor CGM for those with CHI e.g. improve accuracy at lower glucose levels
	Objective evidence of low glucose	Adhesive problems			
	Optimisation of blood glucose control				

CGM's feature of predicting future glucose values was found to be particularly useful to prevent hypoglycaemia: *"because it does kind of alert you if my son's about to have a low and then I can act on that "- P5* Through predictive warnings and optimisation of mealtimes and medication timings, CGM allowed for general and persistent improvement in blood glucose control compared to management pre-CGM. *"Mainly, in my opinion, it [CGM] has helped [patient's name] not get any low sugars and to contain his sugar levels, which, obviously, low sugar levels are not good for you anyway. So, in our opinion, it's helped us not get any low sugars." – P1.*

Educational Tool

CGM was perceived to be an enlightening educational tool; the technology allowed families to obtain new knowledge about glucose trends specific to their child: *"It takes about an hour, just over an hour, for his sugar levels to go up. We used to be under the impression that the [octreotide] injection takes fifteen minutes and that could tell us but that wasn't the case so we realised to pay a lot more attention between feeds" – P1.* Most young patients with CHI are unable to verbalise symptoms of hypoglycaemia and many develop relative hypoglycaemia unawareness through recurrence (26); for these reasons, CGM was described as a *"lifechanger"* as it drew attention to low glucose levels when there were no demonstrable signs of hypoglycaemia. *"If it wasn't for that machine, I wouldn't even know, because my son doesn't even display any symptoms" – P5.*

Parents felt that they had been managing the condition appropriately prior to study participation but were surprised by the unexpected number of hypoglycaemias highlighted by CGM, especially in between the times of their usual fingerprick tests. Having gained the new information from CGM about recurrent hypoglycaemia, families had heightened awareness of low glucose levels at certain times of the day: *"It kind of made you more aware that when you had access to it, you knew that it was going to dip at a certain point and you thought – 'well, he's just played football for an hour and a half, he's going to need something' " – P4.* This allowed parents to reflect and analyse the possible reasons for hypoglycaemia at specific times, prompting preventative action to achieve normoglycaemia.

Behavioural Change

Long-term behavioural change due to CGM was noted in all but one of the families, especially with regards to mealtimes. *"So we would make sure that he had something a bit more sugary in the evening or have a late dinner, just to make up for those late hours in the morning where he's getting those low sugars" – P1. "It showed some certain times I was getting a lower, like, say on a Friday morning, because I start late, I don't get out of bed until later on so I start-my blood was dropping so I then did end up making a slight change to my diet by eating, by making sure I definitely ate the night before and waking up slightly earlier." – P7.*

The timing of medications was not generally changed by families, but there was increased appreciation for ensuring medications were not missed and given on time as it was noted that glucose levels gradually decreased as the time for medication approached. *"I give her medications earlier most of the time. Her normal dose should be at midnight, but I'm really tired most of the night. I can't stay up until then. Normally when I sleep before midnight, chances are, I would miss her midnight dose. And then she would wake up with a low in the morning." – P3.* CGM was also thought to potentially influence dose adjustments as thorough review of glucose trends was undertaken by families and clinicians. *"But there is talks of hopefully dropping the daytime dose of diazoxide. Maybe we wouldn't have been able to do that if it weren't for the Dexcom. We've been able to monitor it more closely. But because we've had*

a couple of lows, they've told us to hold back on it but hopefully soon." - P10.

Pre-CGM, parents described having to persistently remind their older children to check their glucose levels with fingerprick tests and manage their condition effectively. Because of CGM, family dynamics were reported to have changed for the better: *"We are more calm as well, me and her, we don't argue more because our arguments always stem from her testing her blood sugars. So it kind of reduces that as well. It makes it more peaceful." – P3.* CGM use also allowed for adolescents to gain more independence and responsibility as they developed further understanding of their own condition through monitoring of glucose trends, rather than performing fingerprick tests purely because they were told to do so. *"So she has got her snacks with her - if she's gone to another lesson – she knows, right, my sugar's this and, I think, by the time I get to another lesson, it might go down so she's advanced, she's had something to eat. So then it stays really good." – P6.*

Negative Experiences

Although feedback was largely positive, barriers to CGM use were described, such as disruption from alarms, accuracy, sensor insertion and problems with the receiver range.

All participants independently raised the issue of alarms. When using CGM initially, the alarms due to hypoglycaemia caused panic in the parents of younger children. With increased familiarity with CGM, parents would simply check their child's glucose level with a fingerprick test and act accordingly. However, some families expressed frustration at the constant disruptions, especially at night and at school, resulting in an element of alarm fatigue. *"and then when they are actually low, it just-it just went crazy to be honest. We were kind of thinking, to the point where we had to actually turn it off so we could sleep."- P3.* For one adolescent, it seemed CGM audibly distinguished her as different from the rest of her class. She wished to keep her condition private and the alarms accompanying CGM were not discreet in that regard. However, the other adolescents did not acknowledge similar problems at school.

An alarm was also triggered when the receiver was out of range of the sensor, which would occur at a distance of greater than six metres. Adolescents strongly disliked having to always carry the receiver with them and would often forget the receiver, resulting in further frustration from an activated alarm. "*Well, 'cause it's just annoying having to carry, like, a monitor in my pocket, where I have to know where it is" – P12. "Or sometimes she'll go to the toilet and she'll forget to take it with her and it'll beep. And she'll be like 'Oh my god, I'm in the toilet!' – P3.*

Families questioned the accuracy of the CGM readings. A low glucose reading of less than 3.5 mmol/l would trigger an alarm as advised and set by the clinical team. However, a check fingerprick test would typically demonstrate a higher blood glucose level than the CGM value. *"There was a few times where the machine was going low, but actually when we tested it, for [patient's name] it was fine. So yeah, it was a bit of a tricky..like ooh what do you believe?" – P12.* Participants believed the inaccuracy at low glucose levels was the reason for many of the unnecessary alarms during the study period: "*It seems to do that quite a lot where the machine is just beeping, beeping, beeping – you know his glucose levels are 2.4. It's like in my mind that doesn't even make sense because actually well he's on a nighttime feed, he's getting milk, so how can it be?" – P5. "It was, giving us, like, these warnings to say that her blood sugars were low. It wasn't. It was actually not a lot of times. And it kind of was a bit of a nuisance for a while, because it would beep, just unnecessarily" – P3.*

A challenging aspect for the majority of parents with young children was changing the sensor, which took place every eight to ten days. They viewed the sensor changes to be somewhat uncomfortable and frightening for children. *"I would love to have it [CGM] permanently for my son even though he does have that episode of going crazy when I'm changing it and putting it on. It's quite hard for me as well, 'cause he is really, gets himself really worked up. But I'm okay with him getting worked up for those 5 minutes, because of what the machine provides."- P5.*

Parents also reported problems related to the adhesive used to attach the sensor to the body: *"It's really difficult to take off even with the … I bought this 'Zoff' – everybody has tried to use that. It helps but it's still very sticky, which obviously keeps it in place. It's just when we're trying to remove it, it's not too pleasant." – P9.* This was generally apparent in younger children, but the opposite was noted with one adolescent: *"I do think, however, the sticky plaster around it is not strong enough. Especially for [patient's name], 'cause he's hairy."- P13.*

The blinded aspect of the study was understandably not appreciated; adolescents felt it was pointless to carry a device that did not supply immediate glucose readings and parents felt increased anxiety as they had started to rely on the unblinded CGM readings. *"So, it's almost made you a little bit on edge because you're thinking 'Do I test his sugars? I know he said he's alright and he's not had anything to eat, but this time last week when we could see the results, it was saying this and so is it going to be like that? Do I need to check him? Does he feel alright? Does he not feel alright?' " – P4.* However, there was some subtle acknowledgement that families can become fixated on the readings: one parent of an adolescent preferred the CGM readings being blinded to her as she *"wasn't looking at it all the time. You do become a little bit obsessed with it, especially when it's unblinded, because you're just constantly looking at the readings." – P13.*

The families were able to use the CGM device as part of a study and therefore did not require self-payment for device components or consumables. However, they acknowledged the high cost of the supplies would potentially prevent continued usage of the technology. *"To fund it yourself, it's a lot of money. I think it's about £150-200 a month, which is a lot of money." – P5. "We've found it quite more easy but cost-wise it's quite expensive as well." – P6.*

Design Improvements

The families described some improvements they felt could be made before potential widespread usage of the technology for those with CHI.

A short proximity range for data transmission was cited as a problem. This would be aggravated during routine daily activities

such as during sport or when the receiver was accidentally forgotten. The range was identified by adolescents and their parents as one of the main improvements they would like to occur to the existing device. *"Or if he's playing football obviously, we leave it at the halfway line in his bag. Then obviously it doesn't always pick up because you're not within that distance that it should be" – P12. "So he could give it to one of his coaches during a rugby game or rugby training, because he can't physically have it on him when he's tackling. So the range if it was slightly longer, it would be more beneficial for him." - P8.* Parents of younger children bypassed the issue by using a small bag strapped across the child to hold the receiver when the child was at nursery or outside *"yeah, you have to carry it around, that's quite..yeah..not annoying, but, like it could be a bit better. [Patient's name] wears a little pouch around her to carry it around." – P9.*

An alternative design improvement to the issue of receiver range was for the sensor to transmit signals to a mobile phone or a wearable receiver, such as a watch: "*that monitor should be in to like a watch so that they can just wear it, you know, round their wrist. So that's 24 hours with them, the whole day. And they don't take it off."- P6.* It should be noted that this is available for older children and adults, but a separate receiver was used for the first phase of the study.

Families expected CGM interpretation and use to be tailored for those with CHI. They appreciated that CGM had been initially designed for those with type 1 diabetes, however they thought increased utility could be gained from improved accuracy at lower glucose levels and refined predictive warnings based on glucose trends from those with CHI: *"Sometimes it's expecting what it's going to be a blood sugar and obviously they base it on diabetic people. I think they, how the sugar levels go up and down is different than [patient's name] so maybe diabetics shoots very quickly, [patient's name] less so" – P9.*

Participants thought that a smaller sensor could improve the level of comfort. As the CGM sensor was attached to the body (often on the abdominal wall), the sensor could sometimes disrupt sleep and be obstructive for fastening trousers. *"The monitor that we put on the stomach, the CGM thing. I think we wanted it to be a bit smaller. It was quite big. I think if it was a bit more smaller, they would find it a bit comfortable."– P6.*

DISCUSSION

This study is the first qualitative exploration of experiences of CGM amongst families of patients with CHI. The strengths in this study lie in gathering rich data on the experiences of both adolescents and parents of younger children, with matters examined in depth within semi-structured interviews. Furthermore, the analysis was conducted by an investigator without previous involvement in CGM studies in CHI or prior information about the patients, allowing for an inductive coding approach limiting researcher bias. We have observed emergent themes in this study, such as CGM being a catalyst for behavioural change and end users' design improvements of the device, that have not been reported before. This study highlights the importance of incorporating end-user experience in the clinical application of medical devices. While innovations in healthcare and adoption of new technologies such as CGM are welcomed in CHI (11), it should not be assumed that end users will invariably favour CGM over fingerprick tests for glucose monitoring. It is important that user experience is factored in the clinical decision for use of CGM in patients with CHI.

All parents wished to continue to use CGM in the future with the view that the positive aspects, such as continued reassurance, predictive warnings, and less demanding glycaemic management outweighed the disadvantages. Adolescents, however, were more reluctant to carry on using the same version of the device due to problems with range, frustration with alarms and the need to carry a separate receiver.

Families noted that close monitoring and retrospective analysis of glucose trends enhanced their understanding of factors that influence glucose levels at different times of the day. Although parents were surprised that CGM revealed more hypoglycaemic episodes than previously encountered through intermittent fingerprick tests, the new knowledge obtained from CGM allowed for modification of routines to improve management of CHI.

Refinements to the design of CGM were discussed by participants; interestingly, it was clear to families that the system had not been designed for patients with CHI. In keeping with clinician perspectives (27), parents and patients with CHI commented on the need for future work on glucose forecasting algorithms to improve hypoglycaemia accuracy and predictions in CHI.

Participants' experiences with poor accuracy is in keeping with previous studies: flash glucose monitoring systems were found to overestimate glucose levels compared to fingerprick tests (28), whilst CGM measurements, on average, were lower than fingerprick glucose measurements (15). Furthermore, Worth et al. reported a hypoglycaemia sensitivity of 44% with the Dexcom G6 device [unpublished data, in submission]. In response to questionnaires, most of the parents within Vijayanand et al's study reported better sleep (18). In our study, whilst there was increased parental reassurance of normoglycaemia during the night, participants' sleep was often disrupted due to alarms, especially on initial use of CGM.

Whilst parents reported on their experiences and on behalf of their young children, full exploration of children's views was not grasped in this study. This was also the case for two out of the five adolescents who were either unavailable or did not wish to participate in the discussion. The adolescents that did participate were interviewed alongside a parent, which allowed for parental clarification of adolescents' points. However, although every effort was made by the interviewing researcher to fully explore the young people's views, the presence of parents may have unintentionally limited the adolescents' contributions.

One family that withdrew from the previous phase of the study initially agreed to participate in the qualitative study but was subsequently lost to follow up arrangements. Exclusion of this patient may have introduced an element of positive bias into the remainder analysis. Future efforts should actively seek out

those who did not find CGM useful. Interestingly, whilst the other nine out of ten families completed all twelve weeks of the CGM study; at the time of writing, three further patients have since withdrawn from follow-up provision of CGM now that regular expert review of glucose profiles has ceased, suggesting that unsupported CGM at home is not universally popular amongst those with CHI and participating in the parent study may have unintentionally influenced the findings of the analysis.

A nationwide survey is being developed to be distributed to patients with CHI in the UK to gather the views of all families using CGM. This process of methodological triangulation will aim to establish further validity of the findings from the thematic analysis.

In conclusion, the family experience for the use of CGM in CHI was generally positive. CGM allowed families to learn from glucose trends, prompting the prevention of hypoglycaemic episodes with simple routine changes. Whilst CGM increased reassurance and patients had fewer fingerprick tests, participants disliked the receiver proximity range and alarms. Attention to CHI-specific modifications for CGM, such as improved hypoglycaemic accuracy, is needed to enhance the end user experience for this often underserved patient group.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the University of Manchester and the Health Research Authority of the National Health Service (REC reference 07/H1010/88). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the minor(s)' legal guardian/next of kin for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SA, CW, and IB contributed to conception and design of the study. SA conducted the interviews and analysis and wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.894559/full#supplementary-material

REFERENCES

1. Demirbilek H, Hussain K. Congenital Hyperinsulinism: Diagnosis and Treatment Update. *J Clin Res Pediatr Endocrinol* (2017) 9:69–87. doi: 10.4274/jcrpe.2017.S007
2. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ. Therapies and Outcomes of Congenital Hyperinsulinism-Induced Hypoglycaemia. *Diabetes Med* (2019) 36:9–21. doi: 10.1111/dme.13823
3. Yau D, Laver TW, Dastamani A, Senniappan S, Houghton JAL, Shaikh G, et al. Using Referral Rates for Genetic Testing to Determine the Incidence of a Rare Disease: The Minimal Incidence of Congenital Hyperinsulinism in the UK is 1 in 28,389. *PLoS One* (2020) 15:e0228417–e0228417. doi: 10.1371/journal.pone.0228417
4. Avatapalle HB, Banerjee I, Shah S, Pryce M, Nicholson J, Rigby L, et al. Abnormal Neurodevelopmental Outcomes are Common in Children With Transient Congenital Hyperinsulinism. *Front Endocrinol (Lausanne)* (2013) 4:60. doi: 10.3389/fendo.2013.00060
5. Helleskov A, Melikyan M, Globa E, Shcherderkina I, Poertner F, Larsen AM, et al. Both Low Blood Glucose and Insufficient Treatment Confer Risk of Neurodevelopmental Impairment in Congenital Hyperinsulinism: A Multinational Cohort Study. *Front Endocrinol (Lausanne)* (2017) 8:156. doi: 10.3389/fendo.2017.00156
6. Worth C, Hashmi LA, Yau D, Salomon-Estebanez M, Ruiz DP, Hall C, et al. Longitudinal Auxological Recovery in a Cohort of Children With Hyperinsulinaemic Hypoglycaemia. *Orphanet J Rare Dis* (2020) 15:162–2. doi: 10.1186/s13023-020-01438-0
7. Lord K, Radcliffe J, Gallagher PR, Adzick NS, Stanley CA, De León DD. High Risk of Diabetes and Neurobehavioral Deficits in Individuals With Surgically Treated Hyperinsulinism. *J Clin Endocrinol Metab* (2015) 100:4133–9. doi: 10.1210/jc.2015-2539
8. Worth C, Yau D, Salomon Estebanez M, O'Shea E, Cosgrove K, Dunne M, et al. Complexities in the Medical Management of Hypoglycaemia Due to Congenital Hyperinsulinism. *Clin Endocrinol (Oxf)* (2020) 92:387–95. doi: 10.1111/cen.14152
9. Marks BE, Wolfsdorf JI. Monitoring of Pediatric Type 1 Diabetes. *Front Endocrinol (Lausanne)* (2020) 11:128. doi: 10.3389/fendo.2020.00128
10. Klonoff DC, Ahn D, Drincic A. Continuous Glucose Monitoring: A Review of the Technology and Clinical Use. *Diabetes Res Clin Pract* (2017) 133:178–92. doi: 10.1016/j.diabres.2017.08.005
11. Banerjee I, Raskin J, Arnoux J-B, De Leon DD, Weinzimer SA, Hammer M, et al. Congenital Hyperinsulinism in Infancy and Childhood: Challenges, Unmet Needs and the Perspective of Patients and Families. *Orphanet J Rare Dis* (2022) 17:61. doi: 10.1186/s13023-022-02214-y
12. Braune K, Waldchen M, Raile K, Hahn S, Ubben T, Romer S, et al. Open-Source Technology for Real-Time Continuous Glucose Monitoring in the

Neonatal Intensive Care Unit: Case Study in a Neonate With Transient Congenital Hyperinsulinism. *J Med Internet Res* (2020) 22:e21770. doi: 10.2196/21770
13. Beardsall K, Thomson L, Guy C, Iglesias-Platas I, van Weissenbruch MM, Bond S, et al. Real-Time Continuous Glucose Monitoring in Preterm Infants (REACT): An International, Open-Label, Randomised Controlled Trial. *Lancet Child Adolesc Health* (2021) 5:265–73. doi: 10.1016/S2352-4642(20)30367-9
14. Win M, Beckett R, Thomson L, Thankamony A, Beardsall K. Continuous Glucose Monitoring in the Management of Neonates With Persistent Hypoglycemia and Congenital Hyperinsulinism. *J Clin Endocrinol Metab* (2022) 107:e246–53. doi: 10.1210/clinem/dgab601
15. Rayannavar A, Elci OU, Mitteer L, De Leon DD. Continuous Glucose Monitoring Systems: Are They Useful for Evaluating Glycemic Control in Children With Hyperinsulinism? *Horm Res Paediatr* (2019) 92:319–27. doi: 10.1159/000506230
16. Worth C, Harper S, Salomon-Estebanez M, O'Shea E, Nutter PW, Dunne MJ, et al. Clustering of Hypoglycemia Events in Patients With Hyperinsulinism: Extension of the Digital Phenotype Through Retrospective Data Analysis. *J Med Internet Res* (2021) 23:e26957. doi: 10.2196/26957
17. Pasquini T, Mesfin M, Raskin J. *HI Global Registry 2021 Annual Report*. Glen Ridge: Congenital Hyperinsulinism International (2021).
18. Vijayanand S, Stevenson PG, Grant M, Choong CS, Davis EA, Abraham MB. The Utility of Continuous Glucose Monitoring Systems in the Management of Children With Persistent Hypoglycaemia. *J Pediatr Endocrinol Metab* (2021) 34:1567–72. doi: 10.1515/jpem-2021-0414
19. Strauss A, Corbin JM. Basics of Qualitative Research. In: *Grounded Theory Procedures and Techniques*. Thousand Oaks, CA, US: Sage Publications, Inc (1990).
20. Hammarberg K, Kirkman M, de Lacey S. Qualitative Research Methods: When to Use Them and How to Judge Them. *Hum Reprod* (2016) 31:498–501. doi: 10.1093/humrep/dev334
21. Worth C, Nutter P, Dunne M, Salomon-Estebanez M, Banerjee I, Harper S. *Hypo-Cheat's Aggregated Weekly Visualisations of Risk Reduce Real World Hypoglycaemia* . Available at: https://ssrn.com/abstract=4029484.
22. Braun V, Clarke V. Using Thematic Analysis in Psychology. *Qual Res Psychol* (2006) 3:77–101. doi: 10.1191/1478088706qp063oa
23. Smith JA, Shinebourne P. Interpretative Phenomenological Analysis, APA Handbook of Research Methods in Psychology, Vol 2: Research Designs. In: *Quantitative, Qualitative, Neuropsychological, and Biological*. Washington, DC, US: American Psychological Association (2012). p. 73–82.
24. Braun V, Clarke V. *Thematic Analysis: A Practical Guide*. SAGE Publications, Limited (2021). London, UK.
25. Yardley L. Dilemmas in Qualitative Health Research. *Psychol Health* (2000) 15:215–28. doi: 10.1080/08870440008400302
26. Christesen HT, Brusgaard K, Hussain K. Recurrent Spontaneous Hypoglycaemia Causes Loss of Neurogenic and Neuroglycopaenic Signs in Infants With Congenital Hyperinsulinism. *Clin Endocrinol (Oxf)* (2012) 76:548–54. doi: 10.1111/j.1365-2265.2011.04250.x
27. Worth C, Dunne M, Ghosh A, Harper S, Banerjee I. Continuous Glucose Monitoring for Hypoglycaemia in Children: Perspectives in 2020. *Pediatr Diabetes* (2020) 21:697–706. doi: 10.1111/pedi.13029
28. Alsaffar H, Turner L, Yung Z, Didi M, Senniappan S. Continuous Flash Glucose Monitoring in Children With Congenital Hyperinsulinism; First Report on Accuracy and Patient Experience. *Int J Pediatr Endocrinol* (2018) 2018:3–3. doi: 10.1186/s13633-018-0057-2

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Somatostatin receptors in congenital hyperinsulinism: Biology to bedside

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Congenital hyperinsulinism (CHI), although a rare disease, is an important cause of severe hypoglycemia in early infancy and childhood, causing preventable morbidity and mortality. Prompt diagnosis and appropriate treatment is necessary to prevent hypoglycaemia mediated brain damage. At present, the medical treatment of CHI is limited to diazoxide as first line and synthetic somatostatin receptor ligands (SRLs) as second line options; therefore understanding somatostatin biology and treatment perspectives is important. Under healthy conditions, somatostatin secreted from pancreatic islet δ-cells reduces insulin release through somatostatin receptor induced cAMP-mediated downregulation and paracrine inhibition of β- cells. Several SRLs with extended duration of action are now commercially available and are being used off-label in CHI patients. Efficacy remains variable with the present generation of SRLs, with treatment effect often being compromised by loss of initial response and adverse effects such as bowel ischaemia and hepatobiliary dysfunction. In this review we have addressed the biology of the somatostatin system contexualised to CHI. We have discussed the clinical use, limitations, and complications of somatostatin agonists and new and emerging therapies for CHI.

KEYWORDS

congenital hyperinsulinemia, somatostatin, receptor expression, hypoglycemia, insulin, glucagon

Somatostatin perspectives in congenital hyperinsulinism

Congenital hyperinsulinism is a rare disease with a significant genetic component causing unregulated overproduction of insulin through defects in the insulin-releasing pathway. The coupling of insulin secretion to glucose concentration is not tightly regulated, leading to episodes of severe and recurrent hypoglycemia. Several causative mutations in multiple genes have been described to date. The most severe forms are caused by recessive mutations in *ABCC8* and *KCNJ11* coding for the subunits SUR1 and Kir6.2 of the ATP-sensitive K+ (K-ATP) channel on the pancreatic β-cell membrane. Homozygous and compound heterozygous mutations, as well as dominantly inherited mutations in *ABCC8/KCNJ11* cause diffuse CHI, which is often unresponsive to first line diazoxide treatment and therefore suitable for therapy with second line somatostatin receptor ligands (SRLs). In contrast, a subgroup of patients with paternally inherited recessive mutations in *ABCC8/KCNJ11* may have focal disease, potentially curable by surgical excision of a solitary lesion.

Excess insulin in CHI precludes the development of ketones. Therefore, in CHI there is the absence of both glucose and ketones as primary and alternative fuel sources for brain cells, leading to brain damage. In the short term, intravenous high concentration dextrose and in some patients, continuous administration of feeds with a high carbohydrate content are used to prevent hypoglycemia. Excess carbohydrate administration has the propensity to cause obesity and interfere with the normal development of oral feeding. In many such patients, diazoxide is ineffective, resulting in the need for a trial of SRL to reduce excess insulin secretion, before resorting to irreversible sub-total pancreatectomy with consequent development of insulin dependent diabetes and exocrine pancreatic insufficiency.

Biology of the somatostatin system

Somatostatin is a peptide hormone first isolated from the ovine hypothalamus and noted to be a somatotroph release-inhibiting factor (SRIF) (1, 2). Outside the central nervous system, somatostatin is also produced in δ-cells of the pancreas, in close proximity to α- and β-cells (3, 4), setting up opportunities for fine paracrine regulation of both insulin and glucagon secretion (5, 6), Figure 1. In the gut, somatostatin inhibits in particular the secretion of gastrin, secretin, and VIP, delaying gastric emptying. While the tetradecapeptide somatostatin-14 is the prominent isoform in the hypothalamus, in the gut the larger molecule somatostatin-28 is more prevalent. Somatostatin exhibits pleiotropic actions thoughout the body, many of which involve the inhibition of secretion of several hormones, hence its name 'endocrine cyanide' (7).

Somatostatin has a wide range of therapeutic possibilities in different tissues; to capitalize on the diversity of its actions, many stabilized analogs and agonists have been synthesized over the years, extending the half life of somatostatin for sustained treatment effects (8). Octreotide is a first generation SRL, with a greater potency than native somatostatin for the specific somatostatin subtype 2 receptor and has a half-life of 90-120

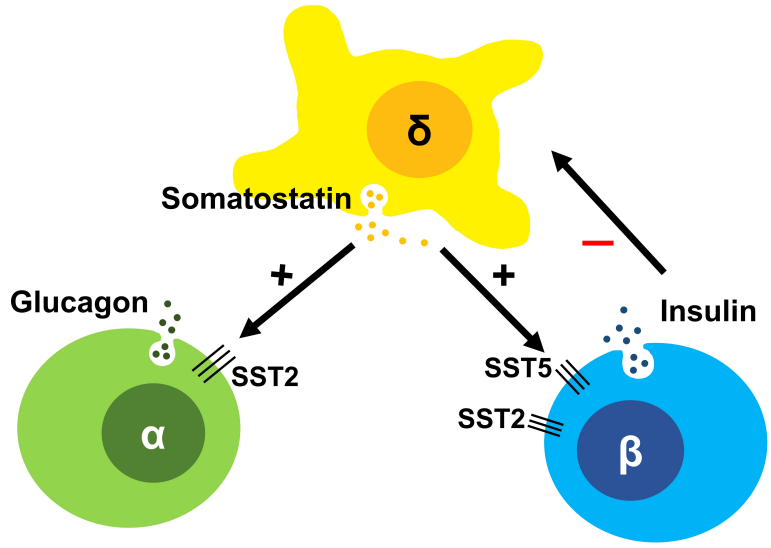

FIGURE 1
The somatostatin induced inhibitory paracrine regulation of the islet system comprising of α, β and δ-cells, with δ-cells inhibiting sustained insulin secretion and so preventing hyperinsulinism in the normal state.

minutes. While short-acting octreotide continues to be used in therapeutic practice in CHI, longer acting depot formulations have been developed, and are approved for the treatment of pituitary and neuroendocrine tumours (5, 9), but increasingly used in the treatment of CHI.

The wide array of effects of somatostatin is mediated by five somatostatin receptor (SST) subtypes (SST1-5), each encoded by a different gene (10). All somatostatin receptors are members of the class-A subgroup of the G-protein coupled receptor (GPCR) superfamily and activate $G_{i/o}$ resulting in inhibition of adenylate cyclase and a decrease in cAMP levels. In response to agonists, SST2 is known to be phosphorylated by G protein receptor kinases (GRKs) and recruits β-arrestins resulting in receptor desensitization and internalization (11). These events and others trigger a range of additional downstream signaling and anti-proliferative effects. However, inhibition of the second messenger cAMP is the primary pathway responsible for its anti-secretory effects. SST2 on islet α-cells suppress glucagon (12), while SST2 and SST5 are primarily responsible for the suppression of insulin in β-cells (6) (Figure 1). In the β-cell, cAMP is hypothesized to be an amplifier of insulin secretion triggered by Ca^{2+} elevation (13); this process is targeted by SRLs to reduce insulin release (Figure 2). It is likely that the somatostatin actions are dependent on the density and distribution of SST receptor subtypes in different tissues (6) as well as variable receptor expression in CHI patients (14).

Intra-islet actions of somatostatin

The human pancreas includes 1-3 million pancreatic islets (15) with a complex interplay between cell types. The secretion of insulin and glucagon by β-cells and α-cells varies reciprocally as a response to plasma glucose levels. Somatostatin-secreting δ-cells are the third most common cell type, representing ~5-10% of islet cells. They possess long neurite-like processes, which can interact with many α- and β-cells (3, 16) making them suitable candidates to exert paracrine regulation (Figure 1).

In isolated human islets, somatostatin is released in response to increasing glucose concentrations (17). The regulation of somatostatin secretion resembles that of insulin secretion from β-cells (18, 19). Like β-cells, δ-cells possess K-ATP channels that close with increasing glucose concentrations resulting in membrane depolarization and somatostatin secretion. These cells similarly respond to sulfonylureas, such as tolbutamide. Indeed diazoxide, which keeps the K-ATP channel complex in the open conformation to prevent depolarization and inhibit insulin secretion in β-cells, also inhibits somatostatin secretion from δ-cells (20). Like other pancreatic hormones, somatostatin is stored temporarily in secretory granules within δ-cells (21) and are released in response to perturbations in inhibitory and excitatory influences.

Endogenous somatostatin is regulated by intra-islet paracrine influences (22) and provides negative feedback to β-cells soon after glucose stimulated insulin release to prevent persistent insulin secretion in physiological states (23–26).

The emerging picture of healthy glucose-insulin secretion coupling is less an isolated β-cell driven action, but more a finely tuned paracrine counterbalance between all islet cell components. In CHI, many of the genes that are mutated in the β-cells of patients are also mutated in both α- and δ-cells. The net effect of the breakdown of this intra-islet homeostasis results in the dysregulation of insulin secretion, leading to significant hypoglycemia (26). More work is needed to unravel how the effects of α-cell and δ-cell dysfunction in CHI pancreatic islets contribute to the overall pathophysiology of CHI and determine individual phenotypes of disease.

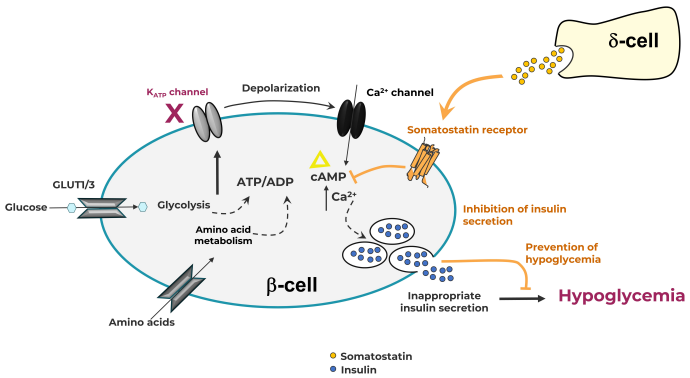

FIGURE 2
The pancreatic β-cell in Congenital Hyperinsulinism showing somatostatin action through G-protein coupled somatostatin receptors on the cell membrane. Elevation of intracellular ATP level drives K-ATP channel closure, membrane depolarization, and subsequent influx of Ca^{++} ions. Increases in intracellular Ca^{++} and cAMP levels lead to the release of insulin. Somatostatin receptor activation induces the formation of G_i-GTP, which inhibits adenylate cyclase, preventing the formation of cAMP, thus reducing insulin secretion.

Somatostatin receptor distribution

A large number of studies have sought to characterize the expression of SSTs in human pancreatic tissues, but cataloging the differences between them is beyond the scope of this review (6, 12, 27, 28). Taken together, somatostatin effects in β-cells are predominantly mediated by SST2, SST5 and perhaps SST1, while the impact of SST3 is unclear. SST4 does not appear to have functional expression in the pancreas. In the α-cell, most histological, expression and functional data points to the conclusion that SST2 is the dominant receptor.

Recent work from islets isolated from CHI patients undergoing pancreatectomy for focal, diffuse, or anomalous CHI (29) suggested less somatostatin activity than matched controls (30). Intriguingly, SST2 was expressed in nearly all CHI patients, while SST5 was expressed less frequently, although one patient with diffuse CHI did not express either SST2 or SST5 (29). This observation needs to be replicated in other cohorts to understand the real frequency of SST receptor expression variation and the potential implication of selective SST expression for optimal treatment of patients. Further, SST receptor downregulation mechanisms need to be explored, with initial work (14) suggesting a role for treatment related expression variability in non-focal CHI.

Somatostatin receptor ligand use in congenital hyperinsulinism

Somatostatin treatment was first described in a child with CHI after 80% pancreatectomy (31), followed by use of a continuous subcutaneous infusion *via* a pump in a 6-month old infant with insulin excess (32). Following initial demonstration of effect and the synthesis of compounds with extended activity, SRLs are now available for many patients with CHI unresponsive to diazoxide. The short acting SRL octreotide has been used in the treatment of CHI (33, 34) as second line therapy over the last two decades to prevent hypoglycemia and subtotal/near-total pancreatectomy. Currently, SRL therapy is not generally used as first line treatment, except in situations where diazoxide treatment is contraindicated or in countries where diazoxide is not readily available.

Short acting SRL treatment

The strategy to use octreotide subcutaneous injections administered every 4 to 8 hours (35) or as continuous infusions (36) brought some benefit in early observational studies, but response to doses up to 40 mcg/kg/day were not clinically effective to prevent pancreatectomy in the majority of patients. However, following wider use, reports of improved outcomes and avoidance of pancreatectomy were noted (37, 38), establishing octreotide as standard, albeit off-label therapy for CHI. Octreotide is now commonly used as subcutaneous bolus injections or by continuous subcutaneous infusions (using insulin pumps) in doses typically ranging from 5 to 40 microgram/kg/day (39). While the use of higher doses have been reported, treatment effect is rare beyond 20 micrograms/kg/day in most patients. For patients requiring frequent injections or higher doses, short-acting octreotide may be substituted by long acting SRL formulations such as octreotide long acting release (e.g. Sandostatin™, Olatuton™) or lanreotide autogel (Somatuline™).

Long acting SRL treatment

Long acting SRLs have been tried in small groups of patients with reported success (40, 41) in obervational studies. They have the advantage of reduced frequency of administration and therefore the potential to improve patient quality of life (42). However, depot injection can be painful and inefficiently dosed, even when administered by trained staff (43). A number of observational studies have reported on different markers of efficacy (44, 45), but in the absence of a comparative or control arm, the efficacy of such long acting SRLs cannot be calibrated to meaningful outcomes such as the achievement of normoglycaemia and harm-free survival. Further, objective assessments of short and long acting SRLs have not been undertaken to appreciate comparative benefits and risks, given the repurposing focus for clinical use outside standardised trial protocols.

Side effects of SRL treatment

Both octreotide and lanreotide possess similar SST receptor pharmacological profiles (primarily SST2 agonists) (Supplementary Table 1) and are therefore expected to have a similar range of both therapeutic and adverse effects. The long acting depot preparations can persist in tissues for up to a month or longer. In young children this might lead to cumulative effects although the extent of accumulation of adverse effects is not known (45).

The utility of octreotide and other SST2 agonists are often complicated by loss of effect with increasing dose in the initial phase of treatment. This downregulation of the SRL dose-response curve is likely to be a consequence of receptor desensitization and internalization, although demonstration of this effect has not been shown in CHI pancreatic tissue. Octreotide and other SRLs have a number of adverse effects including the prolongation of gastrointestinal transit time, abdominal discomfort, and fat malabsorption (46), which could add to feeding problems or require treatment with pancreatic enzymes. Biliary sludging and accumulation into

stones (47) are reported in adults (46) and children (48) although the need for cholecystectomy has not been described.

SRL therapy has been used for many years to reduce growth hormone excess, mainly in adults. As SRL treatment also reduces growth hormone secretion in children, regular auxology follow up in CHI patients is required. While the incidence of growth hormone deficiency following octreotide remains low (47), it is possible and perhaps likely, that this side effect is under-reported in the absence of long-term auxology datasets in CHI cohorts. As growth is an essential component of childhood, careful examination of stature and organ growth needs to be undertaken with the increasing use of long acting SRLs in CHI. Octreotide use in pregnant mothers with CHI also predisposes the fetus to growth restriction, but may represent the only therapeutic option (49) as alternatives such as diazoxide cause unacceptable reduction in placental blood flow.

In newborn babies, particularly those preterm, the risk of necrotizing enterocolitis, possibly arising from reduced splanchnic vascular flow, is significant and can be life threatening (50, 51). Such risk persists beyond the neonatal period, thus necessitating careful review in follow-up of all patients. Although not life threatening, all forms of short and long acting SRLs have the propensity to cause hepatitis (45, 52), an adverse effect that precludes long-term use in the absence of data demonstrating normal hepatic outcomes in later life. Less commonly reported adverse effects include pancreatic exocrine insufficiency (53) and long QT syndrome with potential risk for cardiac arrythmias (54). Octreotide can also cause drug induced pancreatitis (55), an effect that needs to be heeded through stepped down withdrawal of treatment prior to pancreatectomy in CHI patients. Although not widely reported in CHI, octreotide can cause hair loss (56), an effect that might seem trivial but could have significant impact on the psychosocial well-being of older children.

Emerging somatostatin receptor ligands

Several SRLs have been utilised in the treatment of CHI, mainly in those are unresponsive and in those who experience adverse effects from diazoxide treatment. On the whole there is treatment benefit (45) although quantification is imprecise and probably unreliable. Further, loss of initial treatment effect often seen with octreotide is also likely to be present with long acting SRLs, causing later recurrence of hypoglycaemia.

It is likely that SRLs in development for other conditions such as acromegaly [e.g. paltusotine, https://clinicaltrials.gov/ct2/show/NCT04837040] may also be repurposed for use in CHI patients. An example of such a SRL is pasireotide, currently FDA-approved for the treatment of Cushing's disease in adults. In clinical trials in Cushing's disease, treatment with pasireotide resulted in increases in glucose levels, suggesting collateral pancreatic effect (57). Pasireotide possesses a greater potency for stimulated cAMP inhibition of SST5 than octreotide or lanreotide while also possessing some potency for SST2 and SST3 (58) (Supplementary Table 1). Pasireotide has been observed to result in less inhibition of glucagon secretion than octreotide (57), an effect that may be beneficial in the counterregulatory response to hypoglycaemia in CHI patients. Schwetz et al. (59) described the use of pasireotide effectively to control persistent hypoglycemia in an adult patient with CHI like features. Mooij et al. (60) reported on the use of both short-acting pasireotide injections and long-acting pasireotide in an infant with homozygous *ABCC8* mutations; hypoglycaemia frequency improved but was not sufficient to prevent near-total pancreatectomy. Similar to the off-label use of other long acting SRLs, the long term treatment benefit of pasireotide remains to be clarified.

The use of off-label SRLs has prompted the development of new agonists targeting specific SST receptors, in particular SST5. A First in Human study to assess the safety, tolerability, PK, and PD of HTL0030310 compared to pasireotide has been registered in 2019 [https://www.clinicaltrials.gov/ct2/show/NCT03847207] but results are not yet available.

An orally-available selective nonpeptide SST5 agonist, CRN02481, has been shown to suppress insulin secretion and increase glucose levels in both oral glucose tolerance tests and a sulfonylurea model of hyperinsulinism (61, 62). CRN02481 prevented fasting hypoglycaemia and amino acid-stimulated insulin secretion in a $Sur1^{-/-}$ mouse model of CHI (63). Moreover, CRN02481 significantly decreased insulin secretion in human islets isolated from two patients with CHI and one patient with Beckwith-Weideman Syndrome (BWS) CHI, providing ex-vivo demonstration of efficacy in targeted hyperinsulinaemic patients (63).

Conclusions

The biology of SST receptors and ability of SRLs to activate specific receptor subtypes to reduce excess insulin is important in the understanding of the pathobiology and treatment perspectives in CHI. Pharmacological targeting of SST receptors reduces insulin release directly, bypassing K-ATP channel defect dysregulation, thereby providing treatment alternatives to diazoxide-unresponsive CHI patients. Both short and long acting SRLs have been used as second line treatments with similar therapeutic and adverse effect profiles. SRLs with specific β-cell action are currently being developed; these drugs may have improved efficacy and reduced adverse effect profiles, providing much needed therapeutic choice before considering irreversible sub-total pancreatectomy as a last resort for the treatment of severe and recurrent hypoglycaemia.

Author contributions

All authors contributed to the first draft of the manuscript. All authors contributed to the manuscript revision. MA, KM, IB and SB read and approved the submitted version. To our sorrow, MD unexpectedly died during the drafting phase of this manuscript.

Conflict of interest

Author IB is associated with research grants in the development of somatostatin receptor ligands. He is also involved in novel drug development in Congenital Hyperinsulinism. Author SB is an employee of Crinetics Pharmaceuticals, which has an interest in the development of specific forms of somatostatin receptor ligands in Congenital Hyperinsulinism.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.921357/full#supplementary-material

References

1. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, et al. Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. *Science* (1973) 179:77–9. doi: 10.1126/science.179.4068.77

2. Krulich L, Dhariwal AP, McCann SM. Stimulatory and inhibitory effects of purified hypothalamic extracts on growth hormone release from rat pituitary *in vitro*. *Endocrinology* (1968) 83:783–90. doi: 10.1210/endo-83-4-783

3. Cabrera O, Berman DM, Kenyon NS, Ricordi C, Berggren P-O, Caicedo A. The unique cytoarchitecture of human pancreatic islets has implications for islet cell function. *Proc Natl Acad Sci USA* (2006) 103:2334–9. doi: 10.1073/pnas.0510790103

4. Brereton MF, Vergari E, Zhang Q, Clark A. Alpha-, delta- and PP-cells: Are they the architectural cornerstones of islet structure and Co-ordination? *J Histochem Cytochem* (2015) 63:575–91. doi: 10.1369/0022155415583535

5. Cives M, Strosberg J. The expanding role of somatostatin analogs in gastroenteropancreatic and lung neuroendocrine tumors. *Drugs* (2015) 75:847–58. doi: 10.1007/s40265-015-0397-7

6. Strowski MZ, Blake AD. Function and expression of somatostatin receptors of the endocrine pancreas. *Mol Cell Endocrinol* (2008) 286:169–79. doi: 10.1016/j.mce.2008.02.007

7. Bloom SR, Polak JM. Somatostatin. *Br Med J (Clin Res Ed)* (1987) 295:288–90. doi: 10.1136/bmj.295.6593.288

8. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, et al. SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci* (1982) 31:1133–40. doi: 10.1016/0024-3205(82)90087-X

9. Molitch ME. Diagnosis and treatment of pituitary adenomas: A review. *JAMA* (2017) 317:516–24. doi: 10.1001/jama.2016.19699

10. Patel YC, Srikant CB. Somatostatin receptors. *Trends Endocrinol Metab* (1997) 8:398–405. doi: 10.1016/S1043-2760(97)00168-9

11. Hipkin RW, Wang Y, Schonbrunn A. Protein kinase c activation stimulates the phosphorylation and internalization of the sst2A somatostatin receptor. *J Biol Chem* (2000) 275:5591–9. doi: 10.1074/jbc.275.8.5591

12. Singh V, Brendel MD, Zacharias S, Mergler S, Jahr H, Wiedenmann B, et al. Characterization of somatostatin receptor subtype-specific regulation of insulin and glucagon secretion: an *in vitro* study on isolated human pancreatic islets. *J Clin Endocrinol Metab* (2007) 92:673–80. doi: 10.1210/jc.2006-1578

13. Tengholm A, Gylfe E. cAMP signalling in insulin and glucagon secretion. *Diabetes Obes Metab* (2017) 19(Suppl 1):42–53. doi: 10.1111/dom.12993

14. Shi Y, Stevens A, Skae MS, Avatapalle B, Rigby L, Padidela R, et al. Islet somatostatin receptor expression is altered in children with congenital hyperinsulinism (CHI). In: *Hormone research - espe abstract book FC11-182 51* (Basel, Switzerland: Karger Publishers) (2013).

15. Ionescu-Tirgoviste C, Gagniuc PA, Gubceac E, Mardare L, Popescu I, Dima S, et al. A 3D map of the islet routes throughout the healthy human pancreas. *Sci Rep* (2015) 5:14634. doi: 10.1038/srep14634

16. Arrojo E Drigo R, Jacob S, García-Prieto CF, Zheng X, Fukuda M, Tran Thi Nhu H, et al. Structural basis for delta cell paracrine regulation in pancreatic islets. *Nat Commun* (2019) 10:3700. doi: 10.1038/s41467-019-11517-x

17. Ramracheya R, Ward C, Shigeto M, Walker JN, Amisten S, Zhang Q, et al. Membrane potential-dependent inactivation of voltage-gated ion channels in alpha-cells inhibits glucagon secretion from human islets. *Diabetes* (2010) 59:2198–208. doi: 10.2337/db09-1505

18. Ipp E, Dobbs RE, Arimura A, Vale W, Harris V, Unger RH. Release of immunoreactive somatostatin from the pancreas in response to glucose, amino acids, pancreozymin-cholecystokinin, and tolbutamide. *J Clin Invest* (1977) 60:760–5. doi: 10.1172/JCI108829

19. Rorsman P, Huising MO. The somatostatin-secreting pancreatic δ-cell in health and disease. *Nat Rev Endocrinol* (2018) 14:404–14. doi: 10.1038/s41574-018-0020-6

20. Braun M, Ramracheya R, Amisten S, Bengtsson M, Moritoh Y, Zhang Q, et al. Somatostatin release, electrical activity, membrane currents and exocytosis in human pancreatic delta cells. *Diabetologia* (2009) 52:1566–78. doi: 10.1007/s00125-009-1382-z

21. Göpel S, Zhang Q, Eliasson L, Ma X-S, Galvanovskis J, Kanno T, et al. Capacitance measurements of exocytosis in mouse pancreatic alpha-, beta- and delta-cells within intact islets of langerhans. *J Physiol* (2004) 556:711–26. doi: 10.1113/jphysiol.2003.059675

22. Lawlor N, George J, Bolisetty M, Kursawe R, Sun L, Sivakamasundari V, et al. Single-cell transcriptomes identify human islet cell signatures and reveal cell-type-specific expression changes in type 2 diabetes. *Genome Res* (2017) 27:208–22. doi: 10.1101/gr.212720.116

23. Huising MO, van der Meulen T, Huang JL, Pourhosseinzadeh MS, Noguchi GM. The difference δ-cells make in glucose control. *Physiol (Bethesda)* (2018) 33:403–11. doi: 10.1152/physiol.00029.2018

24. DiGruccio MR, Mawla AM, Donaldson CJ, Noguchi GM, Vaughan J, Cowing-Zitron C, et al. Comprehensive alpha, beta and delta cell transcriptomes reveal that ghrelin selectively activates delta cells and promotes somatostatin release from pancreatic islets. *Mol Metab* (2016) 5:449–58. doi: 10.1016/j.molmet.2016.04.007

25. Samols E, Marri G, Marks V. Promotion of insulin secretion by glucagon. *Lancet* (1965) 2:415–6. doi: 10.1016/S0140-6736(65)90761-0

26. Huising MO. Paracrine regulation of insulin secretion. *Diabetologia* (2020) 63:2057–63. doi: 10.1007/s00125-020-05213-5

27. Kaczmarek P, Singh V, Cashen DE, Yang L, Berk S, Pasternak A, et al. Somatostatin receptor subtypes 2 and 5 mediate inhibition of egg yolk-induced gall bladder emptying in mice. *Neurogastroenterol Motil* (2010) 22:204–9, e66. doi: 10.1111/j.1365-2982.2009.01407.x

28. Strowski MZ, Cashen DE, Birzin ET, Yang L, Singh V, Jacks TM, et al. Antidiabetic activity of a highly potent and selective nonpeptide somatostatin receptor subtype-2 agonist. *Endocrinology* (2006) 147:4664–73. doi: 10.1210/en.2006-0274

29. Mitrofanova LB, Perminova AA, Ryzhkova DV, Sukhotskaya AA, Bairov VG, Nikitina IL. Differential morphological diagnosis of various forms of congenital hyperinsulinism in children. *Front Endocrinol (Lausanne)* (2021) 12:710947. doi: 10.3389/fendo.2021.710947

30. Li N, Yang Z, Li Q, Yu Z, Chen X, Li JC, et al. Ablation of somatostatin cells leads to impaired pancreatic islet function and neonatal death in rodents. *Cell Death Dis* (2018) 9:682. doi: 10.1038/s41419-018-0741-4

31. Hirsch HJ, Loo S, Evans N, Crigler JF, Filler RM, Gabbay KH. Hypoglycemia of infancy and nesidioblastosis. studies with somatostatin. *N Engl J Med* (1977) 296:1323–6. doi: 10.1056/NEJM197706092962305

32. Bougnères PF, Landier F, Garnier P, Job JC, Chaussain JL. Treatment of insulin excess by continuous subcutaneous infusion of somatostatin and glucagon in an infant. *J Pediatr* (1985) 106:792–4. doi: 10.1016/S0022-3476(85)80358-9

33. Hindmarsh P, Brook CG. Short-term management of nesidioblastosis using the somatostatin analogue SMS 201-995. *N Engl J Med* (1987) 316:221–2. doi: 10.1056/NEJM198701223160416

34. Delemarre-van de Waal HA, Veldkamp EJ, Schrander-Stumpel CT. Long-term treatment of an infant with nesidioblastosis using a somatostatin analogue. *N Engl J Med* (1987) 316:222–3. doi: 10.1056/NEJM198701223160417

35. Glaser B, Landaw H. Long-term treatment with the somatostatin analogue SMS 201-995: alternative to pancreatectomy in persistent hyperinsulinaemic hypoglycaemia of infancy. *Digestion* (1990) 45(Suppl 1):27–35. doi: 10.1159/000200258

36. Glaser B, Landau H, Smilovici A, Nesher R. Persistent hyperinsulinaemic hypoglycaemia of infancy: long-term treatment with the somatostatin analogue sandostatin. *Clin Endocrinol (Oxf)* (1989) 31:71–80. doi: 10.1111/j.1365-2265.1989.tb00455.x

37. Glaser B, Hirsch HJ, Landau H. Persistent hyperinsulinemic hypoglycemia of infancy: long-term octreotide treatment without pancreatectomy. *J Pediatr* (1993) 123:644–50. doi: 10.1016/S0022-3476(05)80970-9

38. Thornton PS, Alter CA, Katz LE, Baker L, Stanley CA. Short- and long-term use of octreotide in the treatment of congenital hyperinsulinism. *J Pediatr* (1993) 123:637–43. doi: 10.1016/S0022-3476(05)80969-2

39. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ. Therapies and outcomes of congenital hyperinsulinism-induced hypoglycaemia. *Diabetes Med* (2019) 36:9–21. doi: 10.1111/dme.13823

40. Modan-Moses D, Koren I, Mazor-Aronovitch K, Pinhas-Hamiel O, Landau H. Treatment of congenital hyperinsulinism with lanreotide acetate (Somatuline autogel). *J Clin Endocrinol Metab* (2011) 96:2312–7. doi: 10.1210/jc.2011-0605

41. Kühnen P, Marquard J, Ernert A, Meissner T, Raile K, Wannenmacher G, et al. Long-term lanreotide treatment in six patients with congenital hyperinsulinism. *Hormone Res paediatrics* (2012) 78:106–12. doi: 10.1159/000341525

42. Shah P, Rahman SA, McElroy S, Gilbert C, Morgan K, Hinchey L, et al. Use of long-acting somatostatin analogue (Lanreotide) in an adolescent with diazoxide-responsive congenital hyperinsulinism and its psychological impact. *Hormone Res paediatrics* (2015) 84:355–60. doi: 10.1159/000439131

43. Boyd AE, DeFord LL, Mares JE, Leary CC, Garris JL, Dagohoy CG, et al. Improving the success rate of gluteal intramuscular injections. *Pancreas* (2013) 42:878–82. doi: 10.1097/MPA.0b013e318279d552

44. Le Quan Sang K-H, Arnoux J-B, Mamoune A, Saint-Martin C, Bellanné-Chantelot C, Valayannopoulos V, et al. Successful treatment of congenital hyperinsulinism with long-acting release octreotide. *Eur J Endocrinol* (2012) 166:333–9. doi: 10.1530/EJE-11-0874

45. van der Steen I, van Albada ME, Mohnike K, Christesen HT, Empting S, Salomon-Estebanez M, et al. A multicenter experience with long-acting somatostatin analogues in patients with congenital hyperinsulinism. *Hormone Res paediatrics* (2018) 89:82–9. doi: 10.1159/000485184

46. Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ. Octreotide. *N Engl J Med* (1996) 334:246–54. doi: 10.1056/NEJM199601253340408

47. Yorifuji T, Kawakita R, Hosokawa Y, Fujimaru R, Matsubara K, Aizu K, et al. Efficacy and safety of long-term, continuous subcutaneous octreotide infusion for patients with different subtypes of KATP-channel hyperinsulinism. *Clin Endocrinol (Oxf)* (2013) 78:891–7. doi: 10.1111/cen.12075

48. Abernethy LJ, Davidson DC, Lamont GL, Shepherd RM, Dunne MJ. Intra-arterial calcium stimulation test in the investigation of hyperinsulinaemic hypoglycaemia. *Arch Dis Child* (1998) 78:359–63. doi: 10.1136/adc.78.4.359

49. Boulanger C, Vezzosi D, Bennet A, Lorenzini F, Fauvel J, Caron P. Normal pregnancy in a woman with nesidioblastosis treated with somatostatin analog octreotide. *J Endocrinol Invest* (2004) 27:465–70. doi: 10.1007/BF03345293

50. Laje P, Halaby L, Adzick NS, Stanley CA. Necrotizing enterocolitis in neonates receiving octreotide for the management of congenital hyperinsulinism. *Pediatr Diabetes* (2010) 11:142–7. doi: 10.1111/j.1399-5448.2009.00547.x

51. McMahon AW, Wharton GT, Thornton P, de Leon DD. Octreotide use and safety in infants with hyperinsulinism. *Pharmacoepidemiol Drug Saf* (2017) 26:26–31. doi: 10.1002/pds.4144

52. Avatapalle B, Padidela R, Randell T, Banerjee I. Drug-induced hepatitis following use of octreotide for long-term treatment of congenital hyperinsulinism. *BMJ Case Rep* (2012) 2012:1–3. doi: 10.1136/bcr-2012-006271

53. Ros-Pérez P, Golmayo L, Cilleruelo ML, Gutiérrez C, Celaya P, Lacámara N, et al. Octreotide-related exocrine pancreatic insufficiency (EPI) in congenital hyperinsulinism. *J Pediatr Endocrinol Metab* (2020) 33:947–50. doi: 10.1515/jpem-2019-0565

54. Celik N, Cinaz P, Emeksiz HC, Hussain K, Çamurdan O, Bideci A, et al. Octreotide-induced long QT syndrome in a child with congenital hyperinsulinemia and a novel missense mutation (p.Met115Val) in the ABCC8 gene. *Hormone Res paediatrics* (2013) 80:299–303. doi: 10.1159/000354666

55. Wolfe D, Kanji S, Yazdi F, Barbeau P, Rice D, Beck A, et al. Drug induced pancreatitis: A systematic review of case reports to determine potential drug associations. *PloS One* (2020) 15:e0231883. doi: 10.1371/journal.pone.0231883

56. Lami M-C, Hadjadj S, Guillet G. Hair loss in three patients with acromegaly treated with octreotide. *Br J Dermatol* (2003) 149:655–6. doi: 10.1046/j.1365-2133.2003.05478.x

57. de Heide LJM, Laskewitz AJ, Apers JA. Treatment of severe postRYGB hyperinsulinemic hypoglycemia with pasireotide: a comparison with octreotide on insulin, glucagon, and GLP-1. *Surg Obes Relat Dis* (2014) 10:e31-3. doi: 10.1016/j.soard.2013.11.006

58. Hofland LJ, van der Hoek J, Feelders R, van Aken MO, van Koetsveld PM, Waaijers M, et al. The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas *via* somatostatin receptor type 5. *Eur J Endocrinol* (2005) 152:645–54. doi: 10.1530/eje.1.01876

59. Schwetz V, Horvath K, Kump P, Lackner C, Perren A, Forrer F, et al. Successful medical treatment of adult nesidioblastosis with pasireotide over 3 years: A case report. *Medicine* (2016) 95:e3272. doi: 10.1097/MD.0000000000003272

60. Mooij CF, Tacke CE, van Albada ME, Barthlen W, Bikker H, Mohnike K, et al. Pasireotide treatment for severe congenital hyperinsulinism due to a homozygous ABCC8 mutation. *Ann Pediatr Endocrinol Metab* (2021) 26:278–83. doi: 10.6065/apem.2142010.005

61. Sturchler E, Fowler M, Athanacio J, Kredel T, Johns M, Zhao J, et al. Selective nonpeptide somatostatin subtype 5 (sst5) agonists suppress glucose- and sulfonylurea-induced insulin secretion in rats. *J Endocr Soc* (2019) 3:SAT169. doi: 10.1210/js.2019-SAT-169

62. Fowler MA, Zhao J, Sturchler E, Rico E, de Necochea-Campion R, Athanacio J, et al. Discovery and identification of late stage selective nonpeptide somatostatin subtype 5 (sst5) agonists for the treatment of hyperinsulinemic hypoglycemia. *J Endocr Soc* (2020) 4:MON089. doi: 10.1210/jendso/bvaa046.701

63. Juliana C, Chai J, Rico-Bautista EF, Betz S, de Leon DD. A selective nonpeptide somatostatin receptor 5 (sst5) agonist effectively decreases insulin secretion in the KATPHI mouse model. *Diabetes* (2019) 68(Supplement_1):1182–P. doi: 10.2337/db19-1182-P

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Integration of genomic analysis and transcript expression of ABCC8 and KCNJ11 in focal form of congenital hyperinsulinism

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Background: The focal form of CHI is caused by an autosomal recessive pathogenic variant affecting the paternal homologue of genes *ABCC8* or *KCNJ11* and a second somatic event specifically occurring in the affected islet of Langerhans. The approach of this study was to integrate the genetic changes occurring in pancreatic focal lesions of CHI at the genomic and transcriptional level.

Research Design and Methods: Patients receiving therapeutic surgery and with proven *ABCC8* or *KCNJ11* pathogenic variants were selected and analyzed for loss of heterozygosity (LOH), changes in copy number and uniparental disomy (UPD) on the short am of chromosome 11 by molecular microarray analysis and methylation-specific MLPA. Gene expression was analyzed by RT-PCR and Massive Analysis of cDNA Ends (MACE).

Results: Both genes, *ABCC8* and *KCNJ11*, are located in proximity to the Beckwith-Wiedemann (BWS) imprinting control region on chromosome 11p15. Somatic paternal uniparental isodisomy (UPD) at chromosome 11p was identified as second genetic event in focal lesions resulting in LOH and monoallelic expression of the mutated *ABCC8/KCNJ11* alleles. Of five patients with samples available for microarray analysis, the breakpoints of UPD on chromosome 11p were different. Samples of two patients were analyzed further for changes in gene expression. Profound downregulation of growth suppressing genes *CDKN1* and *H19* was detected in focal lesions whereas growth promoting gene *ASCL2* and pancreatic transcription factors of the endocrine cell lineage were upregulated.

Conclusions: Paternal UPD on the short arm of chromosome 11 appears to be the major second genetic event specifically within focal lesions of CHI but no

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common breakpoint for UDP can be delineated. We show for the first time upregulation of growth promoting *ASCL2* (achaete-scute homolog 2) suggestive of a driving factor in postnatal focal expansion in addition to downregulation of growth suppressing genes *CDKN1C* and *H19*.

KEYWORDS

ABCC8, Achaete-scute complex homolog 2, CHI, *KCNJ11*, UPD 11p

Introduction

Congenital hyperinsulinism (CHI) causes persistent hypoglycemia due to uncontrolled insulin secretion in newborns and infants (1, 2). The most severe form of CHI is caused by inactivating pathogenic variants in the *ABCC8* (MIM 600509) and *KCNJ11* (MIM 600937) genes encoding subunits SUR1 and Kir6.2 of the ATP-sensitive K(+) channel (3, 4). This channel is primarily expressed in the pancreas and to a much lesser degree in other tissues. Genetic testing in several larger patient cohorts revealed more than 100 pathogenic variants in these genes. Most of them are small pathogenic variants detectable by standard sequence analysis (5). To improve patient care, genetic testing has now been implemented in clinical management of CHI (6, 7).

Two major clinical forms of CHI are known. The diffuse form affects all ß-cells in the pancreas and is mainly caused by biallelic recessive inheritance of inactivating pathogenic variants and less frequently by dominantly acting pathogenic variants. Medical therapy, frequent feeding and subtotal pancreatectomy are the current treatment regimens for the diffuse form (8). The focal form is characterized by ß-cell hyperplasia in an affected islet of Langerhans within the pancreas. Surgical resection of a focal lesion potentially cures the patient (9, 10). The focal form appears to be caused by an autosomal recessive pathogenic variant affecting the paternal homologue of either the *ABCC8* or *KCNJ11* gene combined with somatic loss of heterozygosity (LOH) in the lesion (11–13).

ABCC8 and *KCNJ11* are neighboring genes and they are located on the short arm of chromosome 11 in region 11p15 proximal to the imprinting region that when disrupted causes imprinting disorders Beckwith-Wiedemann syndrome (BWS) and Russell-Silver syndrome (RSS). The critical genomic imprinting region is responsible for the expression of growth regulatory genes depending on the parental origin (14, 15). The *IGF2* gene (MIM 147470) encoding insulin-like growth factor 2 is expressed from the paternally derived chromosome and functions as growth promoting factor during embryogenesis and fetal development. The non-coding RNA of the *H19* gene (MIM 103280) is expressed from the maternally derived chromosome and it is a negative regulator of *IGF2* and other genes (16–18). Likewise, the *CDKN1C* gene (MIM 600856) encoding the inhibitor of G1 cyclin dependent kinases $p57^{kip2}$, is preferentially expressed from the maternal allele. In BWS the clinical features are variable manifestations of macrosomia, visceromegaly of intra-abdominal organs and additional features including hyperinsulinism in a small amount of patients. However, BWS patients usually do not carry pathogenic variants in either *ABCC8* or *KCNJ11* and the underlying mechanism responsible for hyperinsulinism in these patients is not known. In BWS, expression from the maternal chromosome 11p15.5 is compromised either by imprinting defects or by paternal uniparental isodisomy (UPD) in 25% of patients and copy number variations (CNVs) in 9% of patients (14, 19). The focal form of CHI appears to represent a highly restricted type of UPD11p15 somatic mosaicism. In focal lesions LOH at 11p15 was first described using microsatellites and was later confirmed by loss of the maternal allele (12, 20, 21). Further studies showed an imbalance of gene expression in focal lesions (22).

In this study, we performed an integrative analysis of LOH, copy number changes and methylation at the BWS/RSS region followed by changes in gene expression in focal pancreatic lesions of patients harboring pathogenic variants in *ABCC8* and *KCNJ11*, respectively.

Materials and methods

Patients and pancreatic tissue samples

Patients were from the German Registry for Congenital Hyperinsulinism (23). Written informed consent was obtained from the parents of patients and in accordance to the approval by the local ethics committees. Patients were treated by surgical therapy because of the clinical and genetic indication of focal CHI and localization of lesions by imaging diagnostics (10). Histological examination of resected pancreatic tissue presented with a lobular structure. Focal accumulation of atypical islet cells of Langerhans showed only a small rim of adjacent exocrine parenchyma. The endocrine cells exhibited huge nuclei and a broad eosinophilic

cytoplasm. By immunohistochemistry the endocrine cells showed a strong positive reaction with insulin, whereas no reaction with p57 antibody was observed.

Mutation/variant analysis

Following histological examination of resected pancreatic lesions DNA and RNA was simultaneously extracted from deep frozen tissues using the QIAamp DNeasy Blood & Tissue Kit and QIAmp RNeasy Mini Kit (QIAGEN GmbH, Hilden, Germany).

Pathogenic variants and LOH in genes *ABCC8* and *KCNJ11* were analyzed by PCR amplification of the corresponding exons followed by Sanger sequencing using the Big Dye Terminator Cycle Sequencing kit and ABI 3500XL sequencer (Applied Biosystems, Foster City, USA). Coding SNPs in *ABCC8* (dbSNP, NCBI) rs1799858 (exon 14), rs1805036 (exon 21), rs1799859 (exon 31), rs757110 (exon 33) and in *KCNJ11* rs5213, rs5215, rs5218, rs5219 were also included in LOH analysis.

Sequences were processed by SeqPilot software 4.2.1 (JSI Medical Systems GmbH, Ettenheim, Germany). The sequencing data were compared with reference sequence NM_001287174.1 (ENST00000302539; *ABCC8*) and NM_000525.3 (ENST00000339994; *KCNJ11*).

Methylation-specific MLPA

Methylation-specific (MS) Multiplex Ligation-dependent Probe amplification (MLPA) analysis was carried out using probemix ME030-C3 as described by the manufacturer (MRC Holland, Amsterdam, The Netherlands). Amplification products were identified and quantified by capillary electrophoresis on an ABI 3500XL genetic analyzer. MLPA profiles were analyzed with the module MLPA of SeqPilot (JSI Medical Systems GmbH, Ettenheim, Germany).

Molecular microarray analysis

SNP-based chromosomal microarray (CMA) analysis was performed using a CytoScan™ HD microarray and the Chromosome Analysis Suite v4.3.0.71 (Thermo Fisher Scientific, Waltham, MA USA). All genomic positions were according to the GRCh37/hg19 build of the human reference genome.

RT-PCR, massive analysis of cDNA ends (MACE)

Expression analysis of *ABCC8* and *KCNJ11* was performed by RT-PCR. The same pancreatic tissue samples were examined for genomic and RNA changes. Total RNA was reverse transcribed using the Super ScriptIII™ RT-PCR kit (Invitrogen, Carlsbad, USA) as recommended by the supplier. RT-PCR was performed with primers from the coding regions of the genes using internal exon-spanning primers for *ABCC8*. For the single exon gene *KCNJ11* the RNA was treated with DNAse prior to reverse transcription in order to exclude amplification of residual genomic DNA in the reaction. For controlling specific transcript amplification of *KCNJ11*, coding SNPs of *ABCC8* were analyzed in parallel by exon-spanning RT-PCR. RT-PCR products were analyzed by direct sequencing as outlined above.

MACE was performed on extracted RNA from focal lesions and adjacent pancreatic normal tissue of the same patients (Patient 3 and 8) and expression was compared within the same patient. Data were analyzed as described previously (24) by GenXPro GmbH, Frankfurt a.M., Germany.

Results

Pathogenic variants in K_{ATP}-channel genes *ABCC8* and *KCNJ11*

Paternally transmitted heterozygous pathogenic variants in either *ABCC8* or *KCNJ11* were previously identified during molecular genetic analysis in blood cells of the patients and their parents and have been described by Mohnike et al. (23) and Barthlen et al. (10). Of the 10 patients included in this study, 6 harbored pathogenic variants in *ABCC8* and 4 harbored pathogenic variants in *KCNJ11* (Table 1).

The DNA of pancreatic samples was analyzed for LOH following histological examination of frozen tissue biopsies. Focal lesions and adjacent normal pancreatic tissue if available were examined. LOH analysis performed at the pathogenic variant site in the respective sample in addition to informative intragenic single nucleotide polymorphisms (SNPs) of both, *ABCC8* and *KCNJ11*, genes was close to 100% in 7 samples (Table 1).

Paternal uniparental isodisomy in focal lesions

Copy number-neutral LOH at the *ABCC8/KCNJ11* locus as determined by MLPA suggested uniparental isodisomy (UPD) of chromosomal region 11p15 including the *ABCC8* and *KCNJ11* loci. At both imprinting centers of the BWS/RSS region on 11p15.5, an imbalance in methylation typically found in BWS patients with paternal UPD 11p15 was detected in 7 focal samples (Table 1; Figure 1). In 3 patients (Patient 6, 7, 10) with intermediate LOH in genomic DNA, paternal UPD 11p15 was similar in pattern but differences were less pronounced than in the other samples, thus suggesting a mosaic status for UPD 11p15 in these samples.

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TABLE 1 Genetic and expression analysis of pancreatic lesions from focal CHI.

Patient	Exon	Pathogenic Variant Nucleotide	Protein	Observed freq. [Ref.]	Age at surgery (months)	mRNA expression	LOH	Paternal UPD11p15
ABCC8								
1	1	c.50T>C	p.Val17Ala	2†	10	monoallelic mutant	++	++
2	10	c.1530G>T	p.Lys510Asn	1	10	monoallelic mutant	++	++
3	12	c.1792C>T	p.(Arg598*)	Multiple [CM050968]	7	no (NMD)	++	++
4	34	c.4162_4164delTTC	p.Phe1388del	Multiple [CD962164]	9	monoallelic mutant	++	++
5	35	c.4241C>T	p.Pro1414Leu	Multiple [CM068331]	6	monoallelic mutant	++	++
6	35	c.4259C>T	p.Ser1420Leu	1	2	monallelic mutant	+	+
KCNJ11								
7	1	c.286G>A	p.Ala96Thr	1†	2	mutant/wt 75%/25%	+	+
8	1	c.612C>A	p.Asp204Glu	2 [CM083531]	2	monoallelic mutant	++	++
9	1	c.844G>A	p.Glu282Lys	3 [CM071810]	17	monoallelic mutant	++	++
10	1	c.901C>G	p.Arg301Gly	Multiple [CM088147]	6	monoallelic mutant	(+)	+s

Patients described in Mohnike et al. (23) and Barthlen et al. (10).
Pathogenic variants were of paternal origin and heterozygous in blood or adjacent pancreatic tissue.
ABCC8 RefSeq NM_001287174.1, KCNJ11 RefSeq NM_000525.3.
†ABCC8 c.50T>C was recorded once in ExAC (11:17498274 A/G), allele frequency 1.297e-05; KCNJ11 c.286G>A was recorded once in ExAC (11:17409353 C/T), allele frequency of 8.269e-06 [Ref.] pathogenic variants reported in Human Gene Mutation Database (HGMD).
LOH, loss of heterozygosity; ++, >80-100% loss of the maternal allele; +, >50-80% loss of the maternal allele; (+), >20-50% loss of the maternal allele; UPD 11p15, uniparental isodisomy including paternal imprint; ++, complete pUPD; +, incomplete pUPD.

In DNA from pancreatic tissue specimens of patients 1, 3, 7, 8, and 9 we could demonstrate regions of copy neutral loss of heterozygosity (LOH) on the short arm of chromosome 11, suggesting segmental uniparental disomy (UPD) 11. All samples showed different dimensions of the UPD region with the smallest region in patient 3 (20,3Mb) and the largest region in patient 7 (43,2Mb) (Figure 1 and Table 2). In all patients the *ABCC8* gene is located within the UPD region. Furthermore the samples showed different levels of mosaicism ranging from <50% to >75%.

Monoallelic expression of *ABCC8* and *KCNJ11* transcripts from paternally transmitted mutant alleles

In normal pancreatic tissue biallelic expression of *ABCC8* and *KCNJ11* was found by sequencing and fragment analysis. In the normal pancreatic tissue of patient 3 only the *ABCC8* transcript from the maternal wild-type allele was detectable (Figure 2A). This patient harbors a paternally inherited nonsense pathogenic variant in *ABCC8* resulting in a premature termination codon that is likely to cause nonsense-mediated RNA decay (NMD). By RT-PCR the amount of mutant *ABCC8* transcripts was below limit of detection despite apparent heterozygosity at the DNA level. Accordingly, there are no *ABCC8* transcripts identified beyond background level in the focal lesion when the maternal wild-type allele is lost (Figure 2A, Patient 3). Monoallelic expression of the mutant transcripts encoded by the paternally inherited alleles was observed for all other focal lesions from the remaining patients in this series (Table 1; Figure 2A). Patient sample 7 showed residual expression of 10-25% of the wild-type transcript from the maternal allele concomitant with incomplete LOH in genomic DNA.

Gene expression analysis

Expression analysis for genes located in 11p15 in focal lesions of patient 3 and 8 revealed downregulation of the imprinted gene *H19* (log2: 5.8- and 2.6-fold, resulting from 690 766 and 568 794 reads in non-lesional pancreatic tissue as compared to 12 659 and 93 624 reads in focal lesions, respectively). Likewise, we found for the *CDKN1* gene 50 766 and 27 339 reads in non-lesional pancreatic tissue compared to 2 685 and 11 102 reads in focal lesions of patients 3 and 8, respectively, resulting in changes of log2: 4.2- and 1.3-fold. On the other side, we found for *ASCL2* read numbers of 7 002 and 3 417 in non-lesional pancreatic tissue versus 34 140 and 23 683 reads in focal lesions of patient 3 and 8, respectively (Figure 2C). This suggests a lower expression of *ASCL2* in non-lesional tissue and upregulation of *ASCL2* (achaete-scute homolog 2, MIM 601886) relative expression in focal lesions by more than log2: 2.3- and 2.8-fold, respectively. Upregulated gene expression was also observed for several pancreatic transcription factors involved in differentiation of the endocrine cell lineages of alpha and beta cells in both samples investigated (Figure 2C).

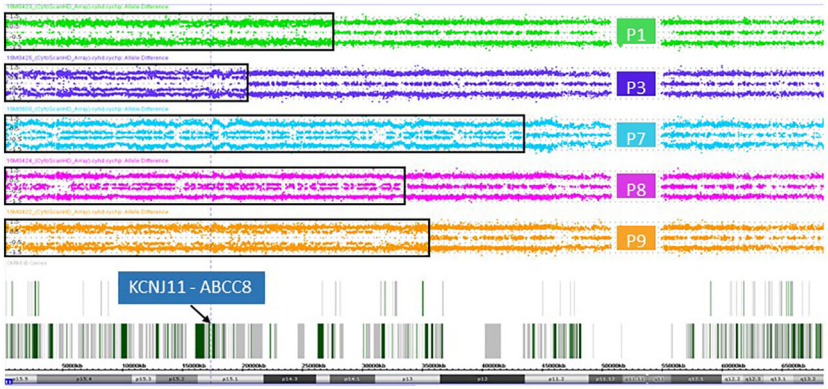

FIGURE 1
Allele difference plots of chromosome 11 on DNA extracted from pancreatic lesions of patient 1 (green panel), patient 3 (purple panel), patient 7 (blue panel), patient 8 (magenta panel) and patient 9 (orange panel) show a mosaic pattern of segmental uniparental disomy (UPD) of the short arm of chromosome 11 of different sizes. The extent of the UPD regions indicated by the black rectangles. The vertical dotted line and the arrow indicates the location of the genes *KCNJ11* and *ABCC8*, which are located within the UPD region in all five patient samples.

Discussion

Our comprehensive molecular analysis of native frozen pancreatic lesions from 10 focal CHI patients revealed monoallelic expression of the K_{ATP}-channel genes *ABCC8* and *KCNJ11* confined to focal lesions. The unaffected pancreatic tissue showed biallelic expression from both parental alleles at about similar ratios providing further evidence that both genes are not subject to parental imprinting in the pancreas. Our results also demonstrate that the recurrent nonsense pathogenic variant c.1792C>T (p.Arg598*) leads to loss of the mutated ABCC8 transcript independent of the second genetic event presumably due to nonsense-mediated mRNA decay [NMD; (25)] as shown for patient 3. Monoallelic expression in focal lesions coincided with LOH at the DNA level. This agrees well with the concept of somatic mosaicism in focal CHI that occurred in an islet by loss of the maternal allele in a progenitor cell and subsequent massive clonal expansion of the progeny cells (22). Incomplete LOH at the DNA level in 3 samples may be explained by admixture of a small cell population with features of non-lesional pancreatic cells residing within that lesion.

In all samples showing LOH, no imbalance in copy numbers was observed on 11p15 by MLPA. This suggests that neither deletion nor duplication at 11p15 is involved in focal CHI. Methylation-sensitive MLPA, however, showed an imprinting pattern of paternal UPD. These results further support the proposed paternal isodisomic UPD 11p15 as the major second genetic event causing LOH in focal CHI (21). Breakpoint-mapping in pancreatic lesions revealed no common breakpoints but encompassed the *ABCC8* and *KCNJ11* genes in the recombination interval as expected (11, 26). Somatic segmental UPD also occurs in many types of cancers and may convey a permissive growth advantage in light of Knudson's two-hit hypothesis. A possible mechanism proposed in formation of segmental UPD in cancerous cells has been mitotic recombination events of homologous non-sister chromatids. Alternatively, an initial deletion may be compensated by re-

TABLE 2 Molecular microarray analysis.

Patient	Karyotype according ISCN2020	Level of mosaicism
1	arr[GRCh37] p15.5p14.1(1_27500235)x2 hmz	> 75%
3	arr[GRCh37] p15.5p15.1(1_20328840)x2 hmz	50-75%
7	arr[GRCh37] p15.5p12(1_43161203)x2 hmz	> 50%
8	arr[GRCh37] p15.5p13(1_33012493)x2 hmz	> 50%
9	arr[GRCh37] p15.5p13(1_35381495)x2 hmz	50-75%

Positions were according to the GRCh37/hg19.

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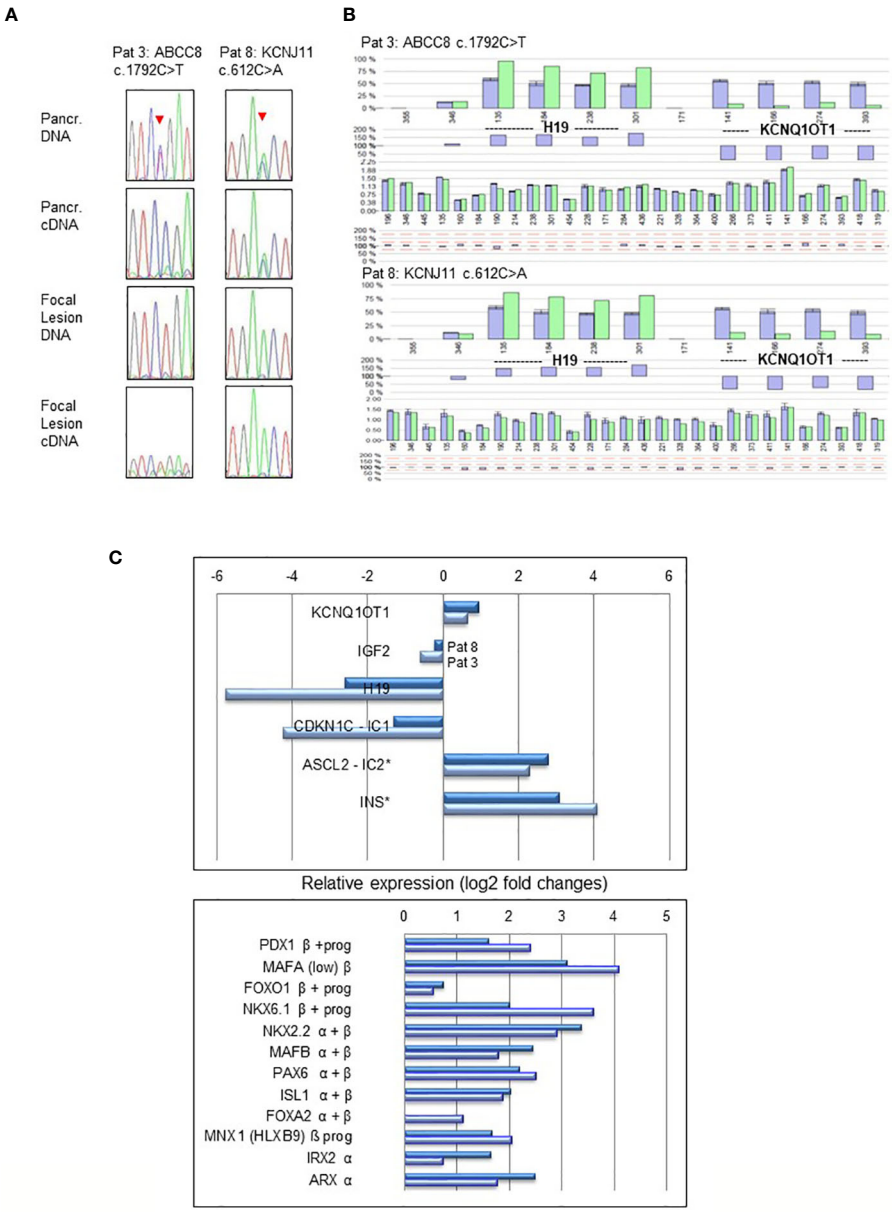

FIGURE 2
LOH and expression analysis performed in pancreatic normal tissue and focal lesions of Patient 3 (*ABCC8* c.1792C>T) and Patient 8 (*KCNJ11* c.612C>A). Heterozygosity of the pathogenic variants and biallelic expression **(A)** is evident in normal pancreatic tissue (upper panel), whereas focal lesions show monoallelic expression and LOH (lower panel). Sequencing profiles show the respective pathogenic variants in *ABCC8* and *KCNJ11* (indicated by an arrowhead) obtained from genomic DNA and reverse transcribed cDNA from focal lesion and pancreatic (Pancr.) normal tissue. In cDNA of Patients 3 pathogenic variant c.1792C>T causes NMD of the transcript resulting in lack of the respective ABCC8 transcripts. Epigenetic and copy number analysis **(B)** at the BWS/RSS imprinting region on 11p15.5 by MS-MLPA demonstrating paternal UPD11p15.5 in pancreatic focal lesions of Patient 3 (*ABCC8* c.1792C>T) and Patient 8 (*KCNJ11* c.612C>A). The pattern of hypermethylation of imprinting center 1 (IC1) at probes for *H19* and hypomethylation of IC2 at probes for *KCNQ1OT1* indicate paternal UPD11p15.5 typically found in BWS with UPD. Dark blue bars are results of three controls compared to the focal DNA (light green bars). Differences of controls and patient DNA shown below each profile. Relative gene expression (log2-fold changes) of BWS/RSS-Region 11p15 (upper panel) and pancreatic transcription factors (lower panel) by MACE analysis in focal lesions of Patient 3 (light blue) and Patient 8 (dark blue) compared to non-lesional tissue of the same patient **(C)**. Genes *ASCL2* located at imprinting center IC2 and *INS* are both not subject to genomic imprinting in humans. Pancreatic transcription factors expressed in progenitors (prog) of the endocrine lineage and in mature α- and β-cells are indicated.

duplication of the homologous region from the remaining chromosomal region of the other chromosome (27). In non-neoplastic tissue, less information exists on tissue-restricted UPD contributing to disease development. The focal form of CHI represents one of few examples of a cell-type restricted segmental paternal UPD 11p resembling a BWS micromosaicism. Based on the prevalence of focal compared to diffuse CHI, the risk of focal CHI in a child who carries a paternally inherited recessive pathogenic variant in *ABCC8* or *KCNJ11* was estimated to be around 1:270 (28). This may suggest that cell and tissue restricted segmental UPD are not rare somatic genetic events in pancreatic and possibly in other tissues. In fact, late onset ß-thalassemia and sickle cell anemia are other diseases with a similar mechanism of mosaic segmental paternal isodisomy at 11p15 unmasking a pathogenic variant in the *HBB* gene followed by clonal selection of hematopoetic progenitor cells due to enhanced proliferation (29, 30).

In focal pancreatic lesions the growth suppressing imprinted genes *H19* and *CDKN1C* located in 11p15 are downregulated, while no substantial change in growth promoting *IGF2* expression was detected. However, we observed upregulation of *ASCL2* (achaete-scute homolog 2) also located in 11p15 but not subject to imprinting in humans. The gene encodes a basic helix-loop-helix transcription factor, which is target of WNT signaling in intestinal stem cells and exerts oncogenic function in cell culture (31). In a mouse model, transgenic rescue of *Ascl2* expression leads to placentomegaly associated with BWS indicating a critical role of *Ascl2* in placental overgrowth (32). Our results suggest that in fact upregulated *ASCL2* is a driver in focus formation in postnatal CHI in addition to downregulated *H19* and *CDKN1*. Currently, it is not known whether downregulated expression of *H19* or additional components are responsible for upregulation of *ASCL2* in focal lesions of the pancreas. Concomitantly, several key transcription factors of the endocrine pancreatic linages including premature stages (33) were upregulated in focal lesions.

Conclusion

In conclusion, our results support the hypothesis of paternal UPD 11p15 in focal CHI that leads to monoallelic expression of the mutated channel genes and appears to be the major second genetic event specifically in the pancreatic endocrine lineage. Clonal expansion of focal lesions appears to be driven by upregulation of growth promoting *ASCL2* (achaete-scute homolog 2) in addition to downregulation of growth suppressing genes *CDKN1* and *H19*.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, upon request.

Ethics statement

The studies involving human participants were reviewed and approved by University Hospital Magdeburg. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

IW, MZ planned this study. IW, IS, IF, SV, MZ performed, analyzed and interpreted the data. WB, KM recruited and collected clinical samples. IW, IS, MZ participated in writing. All authors approved the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Arnoux JB, Verkarre V, Saint-Martin C, Montravers F, Brassier A, Valayannopoulos V, et al. Congenital hyperinsulinism: current trends in diagnosis and therapy. *Orphanet J Rare Dis* (2011) 6:63. doi: 10.1186/1750-1172-6-63

2. Stanley CA, De León DD. Frontiers in diabetes. In: *Monogenic hyperinsulinemic hypoglycemia disorders*, vol. Vol 21. (Basel, CH: Karger) (2012). doi: 10.1159/isbn.978-3-8055-9944-3

3. Thomas PM, Cote GJ, Wohllk N, Haddad B, Mathew PM, Rabl W, et al. Mutations in the sulfonylurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* (1995) 268:426–9. doi: 10.1126/science.7716548

4. Nestorowicz A, Inagaki N, Gonoi T, Schoor KP, Wilson BA, Glaser B, et al. A nonsense mutation in the inward rectifier potassium channel gene, Kir6.2, is associated with familial hyperinsulinism. *Diabetes* (1997) 46:1743–8. doi: 10.2337/diab.46.11.1743

5. De Franco E, Saint-Martin C, Brusgaard K, Knight Johnson AE, Aguilar-Bryan L, Bowman P, et al. Update of variants identified in the pancreatic β-cell K_{ATP} channel genes KCNJ11 and ABCC8 in individuals with congenital hyperinsulinism and diabetes. *Hum Mutat* (2020) 41:884–905. doi: 10.1002/humu.23995

6. Banerjee I, Avatapalle B, Padidela R, Stevens A, Cosgrove KE, Clayton PE, et al. Integrating genetic and imaging investigations into the clinical management of congenital hyperinsulinism. *Clin Endocrinol* (2013) 78:803–13. doi: 10.1111/cen.12153

7. Maiorana A, Barbetti F, Boiani A, Rufini V, Pizzoferro M, Francalanci P, et al. Focal congenital hyperinsulinism managed by medical treatment: A diagnostic algorithm based on molecular genetic screening. *Clin Endocrinol* (2014) 81:679–88. doi: 10.1111/cen.12400

8. Lord K, Dzata E, Snider KE, Gallagher PR, De León DD. Clinical presentation and management of children with diffuse and focal hyperinsulinism: a review of 223 cases. *J Clin Endocrinol Metab* (2013) 98:E1786–1789. doi: 10.1210/jc.2013-2094

9. Laje P, Stanley CA, Palladino AA, Becker SA, Adzick NS. Pancreatic head resection and roux-en-Y pancreaticojejunostomy for the treatment of the focal form of congenital hyperinsulinism. *J Pediatr Surg* (2012) 47:130–5. doi: 10.1016/j.jpedsurg.2011.10.032

10. Barthlen W, Varol E, Emptin S, Wieland I, Zenker M, Mohnike W, et al. Surgery in focal congenital hyperinsulinism (CHI) – the "Hyperinsulinism Germany international" experience in 30 children. *Pediatr Endocrinol Rev* (2016) 14:48–56. doi: 10.17458/PER.2016.BVE

11. Giurgea I, Sempoux C, Bellanné-Chantelot C, Ribeiro M, Hubert L, Boddaert N, et al. The knudson's two-hit model and timing of somatic mutation may account for the phenotypic diversity of focal congenital hyperinsulinism. *J Clin Endocrinol Metab* (2006) 91:4118–23. doi: 10.1210/jc.2006-0397

12. Damaj L, le Lorch M, Verkarre V, Werl C, Hubert L, Nihoul-Fétéké C, et al. Chromosome 11p15 paternal isodisomy in focal forms of neonatal hyperinsulinism. *J Clin Endocrinol Metab* (2008) 93:4941–7. doi: 10.1210/jc.2008-0673

13. Gillis D. Familial hyperinsulinism. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. *GeneReviews® [Internet]*. (Seattle, USA: University of Washington) (2019). p. 1993–2020.

14. Feinberg AP. Phenotypic plasticity and epigenetics of human disease. *Nature* (2007) 447:433–40. doi: 10.1038/nature05919

15. Demars J, Gicquel C. Epigenetic and genetic disturbance of the imprinted 11p15 region in beckwith-wiedemann and silver-russel snydromes. *Clin Genet* (2012) 81:350–61. doi: 10.1111/j.1399-0004.2011.01822.x

16. Gabory A, Ripoche M-A, Le Digarcher A, Watrin F, Ziyyat A, Forné T, et al. H19 acts as a trans regulator of the imprinted gene network controlling growth in mice. *Development* (2009) 136:3413–21. doi: 10.1242/dev.036061

17. Monnier P, Martinet C, Pontis J, Stancheva I, Ait-Si-Ali S, Dandolo L. H19 lncRNA controls gene expression of the imprinted gene network by recruiting MBD1. *Proc Natl Acad Sci USA* (2013) 110:20693–8. doi: 10.1073/pnas.1310201110

18. Wang B, Suen CW, Ma H, Wang Y, Kong L, Qin D, et al. The roles of H19 in regulating inflammation and aging. *Front Immunol* (2020) 11:579687. doi: 10.3389/fimmu.2020.579687

19. Begemann M, Spengler S, Gogiel M, Grasshoff U, Bonin M, Betz R. Clinical significance of copy number variations in the 11p15.5 imprinting control regions: new cases and review of the literature. *J Med Genet* (2012) 49:547–53. doi: 10.1136/jmedgenet-2012-100967

20. De Lonlay P, Fournet JC, Rahier J, Gross-Morand MS, Poggi-Travert F, Foussier V, et al. Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. *J Clin Invest* (1997) 100:802–7. doi: 10.1172/JCI119594

21. Verkarre V, Fournet JC, de Lonlay P, Gross-Morand MS, Devillers M, Rahier J, et al. Paternal mutation of the sulfonylurea receptor (SUR1) gene and maternal loss of 11p15 imprinted genes lead to persistent hyperinsulinism in focal adenomatous hyperplasia. *J Clin Invest* (1998) 102:1286–91. doi: 10.1172/JCI4495

22. Fournet JC, Mayaud C, de Lonlay P, Gross-Morand MS, Verkarre V, Castanet M, et al. Unbalanced expression of 11p15 imprinted genes in focal forms of congenital hyperinsulinism: association with a reduction to homozygosity of a mutation in ABCC8 or KCNJ11. *Am J Pathol* (2001) 158:2177–84. doi: 10.1016/S0002-9440(10)64689-5

23. Mohnike K, Wieland I, Barthlen W, Vogelgesang S, Empting S, Mohnike W, et al. Clinical and genetic evaluation of patients with K_{ATP} channel mutations from the German registry for congenital hyperinsulinism. *Horm Res Paediatr* (2014) 81:156–68. doi: 10.1159/000356905

24. Zawada AM, Rogacev KS, Müller S, Rotter B, Winter P, Fliser D, et al. Massive analysis of cDNA ends (MACE) and miRNA expression profiling identifies proatherogenic pathways in chronic kidney disease. *Epigenetics* (2014) 9:161–72. doi: 10.4161/epi.26931

25. Popp MW, Maquat LE. Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu Rev Genet* (2013) 47:139–65. doi: 10.1146/annurev-genet-111212-133424

26. Lee BH, Lee J, Kim J-M, Kang M, Kim G-H, Choi J-H, et al. Three novel pathogenic mutations in KATP channel genes and somatic imprinting alterations of the 11p15 region in pancreatic tissue in patients with congenital hyperinsulinism. *Horm Res Paediatr* (2015) 83:204–10. doi: 10.1159/000371445

27. Makishima H, Maciejewski JP. Pathogenesis and consequences of uniparental disomy in cancer. *Clin Cancer Res* (2011) 17:3913–23. doi: 10.1158/1078-0432.CCR-10-2900

28. Glaser B, Blech I, Krakinovsky Y, Ekstein J, Gillis D, Mazor-Aronovitch K, et al. ABCC8 mutation allele frequency in the ashkenazi Jewish population and risk of focal hyperinsulinemic hypoglycemia. *Genet Med* (2011) 13:891–4. doi: 10.1097/GIM.0b013e31821fea33

29. Badens C, Mattei MG, Imbert AM, Lapouméroulie C, Martini N, Michel G, et al. A novel mechansims for thalassemia intermedia. *Lancet* (2002) 359:132–3. doi: 10.1016/s0140-6736(02)07338-5

30. Vinatier I, Martin X, Costa JM, Bazin A, Giraudier S, Joly P. A late onset sickle cell disease reveals a mosaic segmental uniparental isodisomy of chromosome 11p15. *Blood Cells Mol Dis* (2015) 54:53–5. doi: 10.1016/j.bcmd.2014.07.021

31. Jubb AM, Chalasani S, Frantz GD, Smits R, Grasch HI, Kavi V, et al. Achaete-scute like 2 (ascl2) is a target of wnt signaling and is upregulated in intestinal neoplasia. *Oncogene* (2006) 25:3445–57. doi: 10.1038/sj.onc.1209382

32. Tunster SJ, Van de Pette M, Creeth HDJ, Lefebvre L, John RM. Fetal growth restriction in a genetic model of sporadic beckwith-wiedemann syndrome. *Dis Model Mech* (2018) 11:pii: dmm035832. doi: 10.1242/dmm.035832

33. Jennings RE, Berry AA, Strutt JP, Gerrard DT, Hanley NA. human pancreas development. *Development* (2015) 142:3126–37. doi: 10.1242/dev.120063

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Syndromic forms of congenital hyperinsulinism

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Congenital hyperinsulinism (CHI), also called hyperinsulinemic hypoglycemia (HH), is a very heterogeneous condition and represents the most common cause of severe and persistent hypoglycemia in infancy and childhood. The majority of cases in which a genetic cause can be identified have monogenic defects affecting pancreatic β-cells and their glucose-sensing system that regulates insulin secretion. However, CHI/HH has also been observed in a variety of syndromic disorders. The major categories of syndromes that have been found to be associated with CHI include overgrowth syndromes (e.g. Beckwith-Wiedemann and Sotos syndromes), chromosomal and monogenic developmental syndromes with postnatal growth failure (e.g. Turner, Kabuki, and Costello syndromes), congenital disorders of glycosylation, and syndromic channelopathies (e.g. Timothy syndrome). This article reviews syndromic conditions that have been asserted by the literature to be associated with CHI. We assess the evidence of the association, as well as the prevalence of CHI, its possible pathophysiology and its natural course in the respective conditions. In many of the CHI-associated syndromic conditions, the mechanism of dysregulation of glucose-sensing and insulin secretion is not completely understood and not directly related to known CHI genes. Moreover, in most of those syndromes the association seems to be inconsistent and the metabolic disturbance is transient. However, since neonatal hypoglycemia is an early sign of possible compromise in the newborn, which requires immediate diagnostic efforts and intervention, this symptom may be the first to bring a patient to medical attention. As a consequence, HH in a newborn or infant with associated congenital anomalies or additional medical issues remains a differential diagnostic challenge and may require a broad genetic workup.

KEYWORDS

congenital hyperinsulinism, hyperinsulinemic hypoglycemia, Beckwith-Wiedemann syndrome, Sotos syndrome, Costello syndrome, Kabuki syndrome, chromosomal disorders

1 Introduction

Congenital hyperinsulinism (CHI), also termed congenital hyperinsulinemic hypoglycemia (HH), is a disorder of glucose homeostasis due to dysregulated insulin secretion in the newborn or young infant and represents the most common cause of severe and persistent hypoglycemia in infancy and childhood (1). The presenting symptom of

CHI is persistent hypoglycemia typically manifesting shortly after birth with inappropriate insulin levels or indirect signs of inappropriate insulin action such as low plasma concentrations of ketone bodies and free fatty acids, as well as a positive glycemic response to glucagon at the time of hypoglycemia (2). Glucose utilization is increased and leads to high glucose infusion rates required to maintain euglycemia. Affected newborns typically have a high birth weight for gestational age, as fetal insulin secretion promotes *in utero* growth *via* insulin-like growth factor 1 receptor-mediated signaling (3).

CHI is clinically and pathogenetically very heterogeneous. It may be transient and permanent and has a strong genetic contribution. The majority of cases in which a genetic cause can be identified have monogenic defects affecting pancreatic β-cells and their glucose-sensing system that regulates insulin secretion. Such cases typically have a non-syndromic clinical constellation, i.e., no other primary organ manifestations besides the metabolic-endocrine abnormality. Alterations in the genes ABCC8 and KCNJ11 encoding components of the voltage-dependent K_{ATP} channel predominate. They can lead to either diffuse forms of pancreatic involvement due to recessive and dominant mutations, or to focal CHI caused by a unique mechanism involving a heterozygous loss-of-function variant on the paternal allele plus loss of heterozygosity of the 11p15.5 region encompassing the ABCC8/KCNJ11 locus due to a second somatic event (mostly a paternal uniparental disomy of 11p, patUPD11p) in the focal lesion (4). The cause of CHI still remains unknown in up to 50% of patients, which may be due to additional hitherto unidentified genetic loci for monogenic types of CHI (5), as well as the contribution of complex/multifactorial etiologies. In a small fraction of cases, CHI has found to be associated with syndromic or multisystemic diseases (reviewed by 5-8). Few of these syndromic disorders have been associated with genes playing a known direct role in the regulation of glucose metabolism, while for the majority of them the molecular mechanism leading to inappropriately elevated insulin secretion is still unclear. This article reviews syndromic disorders that have been asserted by the literature to be associated with CHI/HH. An overview of disease categories and individual conditions is presented in Table 1. We assess the evidence of the association, as well as the frequency of CHI, its epigenetic and genomic basis, possible pathophysiology and natural course in the respective conditions.

2 Methods

We conducted a systematic PubMed search for original studies and case reports, to identify original data on published syndromic cases of CHI regarding clinical presentation, genetic basis, pathophysiology, diagnosis, and management. General searches were performed using the search term combinations: ("congenital hyperinsulinism") AND (syndrome OR syndromic), as well as: ("congenital hyperinsulinism") AND (Review[Publication Type]), yielding 177 and 183 results, respectively. A separate search was performed for each CHI-associated entity identified through the retrieved original articles and reviews. The search term combination used were: "congenital hyperinsulinism" OR "neonatal hyperinsulinism" OR (hypoglycemia AND insulin) OR hyperinsulinemia OR hyperinsulinemic together with disease-specific terms. For each entity the search terms included disease name and name of disease-causing genes; as an example, the search regarding Sotos syndrome was: ((congenital hyperinsulinism) OR (neonatal hyperinsulinism) OR (hypoglycemia AND insulin) OR (hyperinsulinemia) OR (hyperinsulinemic)) AND (("Sotos syndrome") OR (NSD1)). The reference lists of the identified papers were also used to identify further papers of interest. The final reference list was selected on the basis of relevance to this study. A total of 1144 articles were retrieved by the various searches (excluding the search regarding PI3K-AKT pathway disorders that are not associated with hyperinsulinemia); 176 were selected for data collection. Duplicates of reported cases were excluded, if prior publication in another selected reference was stated by authors. From 27 references where full-text articles were not accessible, available data were extracted from abstracts. The full lists of references and summaries of collected data are presented in Supplementary Table S1.

3 Results

3.1 Beckwith-Wiedemann syndrome

The characteristic clinical features of BWS (OMIM #130650) include fetal/neonatal macrosomia, macroglossia that is often asymmetric, hemihyperplasia, omphalocele or umbilical hernia, visceromegaly involving liver, spleen, kidneys, adrenal glands, and/or pancreas, as well as a predisposition to embryonal tumors (6, 7). In typical cases the diagnosis can quite easily be suspected clinically, based on the combination of characteristic symptoms as mentioned above and other minor anomalies such as ear lobe creases and/or posterior helical ear pits, facial anomalies, nevus flammeus and others. However, BWS represents a clinical spectrum, and some affected newborns may only have few or even singular suggestive clinical findings (7). Most cases are sporadic, but a positive family history is present in about 15% of cases (6). The reported prevalence of BWS is ~1:10,000 live births (8). BWS is caused by epigenetic or genomic alterations leading to abnormal methylation at a distinct differentially methylated region in 11p15.5 (BWS critical region, Figure 1A), namely (i) loss of methylation of IC2 (imprinting center 2) on the maternal chromosome (~50%), (ii) gain of methylation of IC1 on the maternal chromosome (~5%), paternal uniparental disomy of 11p15.5 (patUPD11p; ~20%), or a heterozygous pathogenic variant on the maternal CDKN1C allele (~5%). Other genomic variants involving the chromosome 11p15.5 region including (micro)duplications, (micro)deletions, inversions or translocations account for a small fraction of cases (~1%). BWS-associated epigenetic and genomic changes may be mosaic due to postzygotic occurrence of the underlying (epi)mutation (6, 7). Mosaicism has to be considered particularly in oligosymptomatic cases and its demonstration may be challenging, requiring molecular studies in additional tissues (e.g., skin biopsy from the

TABLE 1 Summary on syndromic diseases having suspected of proven association with CHI/HH.

Disease category	Disease	Inheritance	Locus	Involved gene(s)	Key features	Prevalence of neonatal hypoglycemia	CHI/HH association	Treatment and response	Course and outcome
Overgrowth syndromes	Beckwith-Wiedemann syndrome	Sporadic, AD	11p15.5	Genes of the 11p15.5 DMR; KCNQ1	Hemihyperplasia, macroglossia, omphalocele, visceromegaly	~50%	***patUPD11p***: >30%; few cases with additional K_{ATP} mutation	Often (70%) unresponsive to DXZ; almost half of reported cases received pancreatectomy	Most cases with persistent HI
							Epimutations: <5%	DXZ-reponsive in cases requiring treatment	Most cases with transient HI
	Sotos syndrome	AD	5q35.3	NSD1	Macrosomia, macrocephaly, DD/ID, dysmorphic features	<15%	Very rare, 25 cases reported	DXZ-reponsive; no case of pancreatectomy reported	Transient HI; mostly resolving within the first months of life
	Simpson-Golabi-Behmel syndrome	XL	Xq26.2	GPC3	Macrosomia, DD/ID, dysmorphic features	Increased, not specified	Not documented	NA	NA
	Weaver syndrome	AD	2q27.1	EZH2	Macrosomia, DD/ID, dysmorphic features	Increased, not specified	Not documented	NA	NA
	Perlman syndrome	AR	2q27.1	DIS3L2	Neonatal macrosomia, MCA, nephroblastomatosis, dysmorphic features	Several cases reported	Not documented	NA	NA
	PI3K-AKT pathway disorders	Sporadic, AD	19q13.2, 3q26.3, 1q43-q44, 14q32.33, 12p13.32	AKT2, PIK3CA, AKT3, AKT1, CCND2	Regional overgrowth, vascular malformations, epidermal nevi	Increased frequency of hypoglycemia in infancy and thereafter, not specified	Hypoinsulinemic hypoglycemia (CHI phenocopy), predominantly with AKT2	Unresponsive to DXZ and octreotide; may be managed with regular carbohydrate feeds; response to sirolimus reported	Variable course; spontaneous remission reported
Monogenic developmenttal disorders	Kabuki syndrome	AD	12q13.12, Xp11.3	KMT2D, KDM6A	DD/ID, dysmorphic features, CHD, MCA, postnatal growth defect	<7%	<2%, predominantly with KDM6A	Usually DXZ-responsive; few cases of pancreatectomy reported	Variable course; may require treatment for several years
	Costello syndrome	AD	11p15.5	HRAS	Dysmorphic features, CHD, DD/ID, postnatal growth defect	~44%	Rare	Usually DXZ-responsive; one case of pancreatectomy reported	Persistence up to 6 months reported
	Rubinstein-Taybi syndrome	AD	16p13.3, 22q13.2	CREBBP, EP300	DD/ID, dysmorphic features, MCA, growth defect	Increased, not specified	Very rare	Limited data; more than half of patients responded to DXZ	Variable course; may require treatment for several years
	Coffin-Siris syndrome	AD	Multiple (12)	Multiple (12)	DD/ID, dysmorphic features, MCA	Anecdotal reports	Few cases reported	NA	NA
	CHARGE syndrome	AD	8q12.2	CHD7	MCA, CHD, dysmorphic features, DD/ID	Anecdotal reports	Only 2 cases reported	NA	NA

(Continued)

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TABLE 1 Continued

Disease category	Disease	Inheritance	Locus	Involved gene(s)	Key features	Prevalence of neonatal hypoglycemia	CHI/HH association	Treatment and response	Course and outcome
	FOXA2-CHI	AD	20p11.21	FOXA2	Hypopituitarism, MCA, DD/ID, dysmorphic features	Few cases reported	Few cases reported	Limited data; (partial) response to DXZ	Mostly transient; may be followed by impaired glucose tolerance and diabetes
	MEHMO syndrome	XL	Xp22.11	EIF2S3	DD/ID, hypopituitarism, epilepsy, obesity	Several cases reported	Few cases reported	Limited data; DXZ may improve glycemic response	Hyperinsulinemic hypoglycemia and postprandial hyperglycemia, diabetes may emerge
	Congenital central hypoventilation syndrome	AD	4p13	PHOX2B	Central hypoventilation, Hirschsprung disease	Episodic hypoglycemia reported in several cases	Several cases reported	Limited data; response to DXZ reported	Patients may exhibit postprandial hyperglycemia followed by (asymptomatic) hypoglycaemia
	CHI with renal tubular and hepatic dysfunction	AD	20q13.12	HNF4A (p.R76W)	Renal Fanconi syndrome, hepatic dysfunction	Probably >50%	Several cases reported	Limited data; mostly responsive to DXZ	Mostly transient; may be followed by impaired glucose tolerance and diabetes
	Schaaf-Yang syndrome	AD	15q11.2	MAGEL2	DD/ID, contractures, dysmorphic features	Unknown	Two unrelated observations	Limited data; variable response to DXZ	Few data; DZX treatment up to age 6 reported
Chromosomal (contiguous gene) syndromes	9p deletion syndrome	Sporadic, AD	9p24.1-24.2	SMARCA2, RFX3 (?)	DD/ID, dysmorphic features, MCA	Unknown	Several cases reported	DZX-responsive	Variable course; may require treatment for several years
	Turner syndrome	Sporadic	Xp	KDM6A (?)	Growth defect, hypogonadism, dysmorphic features	Increased, not specified	Several cases reported	Variable response to DXZ; 4 cases with pancreatectomy	Variable course; may require treatment for several years
	Usher-CHI syndrome	AR	11p15.1	ABCC8	Sensorineural deafness, retinitis pigmentosa	100%	100%	DXZ-resistant; most cases received pancreatectomy	Persistent HI
	Trisomy 13	Sporadic	13	Unknown	MCA, dysmorphic features, severe DD/ID	Unknown	Few cases reported	Variable response to DZX	Outcome dictated by underlying disease
	16p11.2 microdeletion	AD	16p11.2	Unknown	Non-specific DD/ID	Unknown	Very rare; few cases reported	Limited data; may respond to DZX	Limited data
	Trisomy 21	Sporadic	21	Unknown	Dysmorphic features, MCA, DD/ID	Unknown	Increased frequency in cohorts tested for CHI	Limited data; mostly DZX-responsive; 1 case of pancreatectomy	Mostly transient; remission within the first year

(Continued)

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TABLE 1 Continued

Disease category	Disease	Inheritance	Locus	Involved gene(s)	Key features	Prevalence of neonatal hypoglycemia	CHI/HH association	Treatment and response	Course and outcome
Channelopathies	Timothy syndrome	AD	12p13.33	CACNA1C	CHD, long-QT syndrome, syndactyly, DD/ID, dysmorphic features	Intermittent hypoglycemia in ~40%	Not documented	Limited data; may respond to DZX	Recurrent episodes of hypoglycemia
	PASNA	AD	3p21.1	CACNA1D	Aldosteronism, epilepsy, hypotonia, DD/ID	Increased, not specified	Two cases reported	Limited data; may respond to DZX or Ca2+ channel blockers	Limited data; DZX treatment up to age 4 reported
	Long-QT syndrome	AD	11p15.5, 7q36.1	KCNQ1, KCNH2	QTc prolongation, cardiac arrhythmia	Intermittent hypoglycemia reported	Hyperinsulinemia after glucose challenge	Limited data; usually no medical treatment required	Postprandial hyperinsulinemia and reactive hypoglycemia
Metabolic disorders	CDG syndrome	AR	16p13.2, 15q24.2, 3q27.1, 1p31.3	PMM2, MPI, ALG3, PGM1	DD/ID, brain and ocular anomalies, hepatopathy, enteropathy, coagulopathy, cystic kidneys	<10-89%, depending on CDG type; PMM2-HI: separate clinical and genetic subtype	Several cases reported	Usually DZX-responsive; may also improve with oral mannose; 1 case of pancreatectomy;	Variable course; may require prolonged DZX treatment
	Tyrosinemia	AR	15q25.1	FAH	Hepatic dysfunction, renal tubular dysfunction, DD/ID	Occasional	Few cases reported	DZX-responsive; specific treatment with NTBC	Mostly transient; dependent on metabolic control
	Adenosine kinase deficiency	AR	10q22.2	ADK	DD/ID, epilepsy, hypermethioninemia, dysmorphic features, CHD	Increased, not specified	Few cases reported	DZX-responsive; specific treatment with methionine restriction	Mostly transient; dependent on metabolic control

CHD, congenital heart defect; CHI, congenital hyperinsulinism; DD/ID, developmental delay/intellectual disability; DMR, differentially methylated region; DZX, diazoxide; HH, hyperinsulinemic hypoglycemia; HI, hyperinsulinism; MCA, multiple congenital anomalies; NA, no data available.

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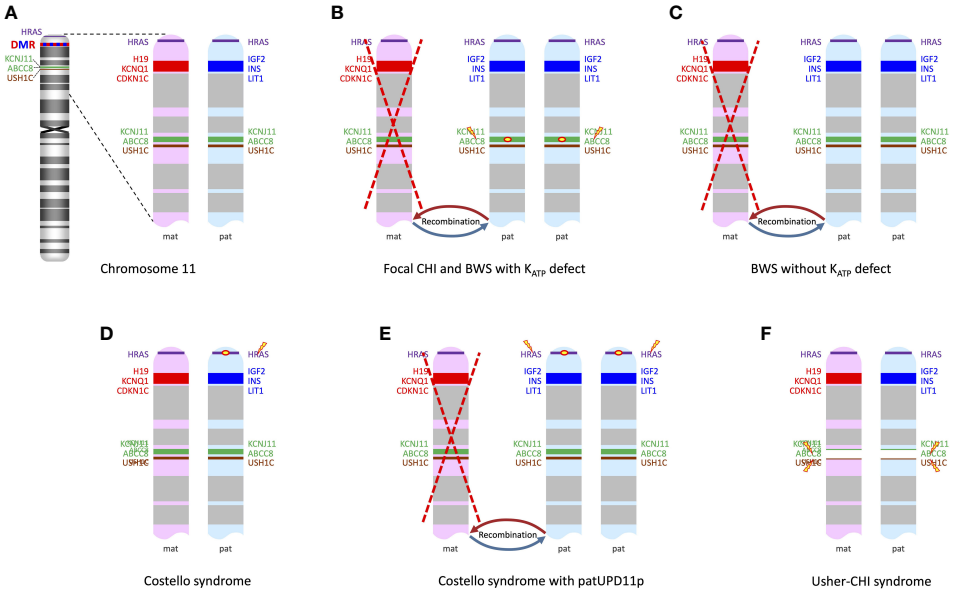

FIGURE 1
(A) ideogram of chromosome 11 displaying the loci of genes of interest in the context of syndromic CHI and the differentially methylated region (DMR) in the 11p15 region; enlarged view of 11p13-11p15 shows genes expressed from the maternal chromosome (in red) and those expressed from the paternal chromosome (in blue). **(B)** patUPD11p with a pathogenic variant in ABCC8 or KCNJ11 on the paternal allele leads to a biallelic defect of ABCC8 or KCNJ11 and at the same time to overexpression of paternally imprinted genes IGF2, INS and LIT1; this mechanism is shared by focal CHI and rare cases of BWS with an ABCC8 or KCNJ11 mutation on the paternal allele. **(C)** BWS due to patUPD11p without an ABCC8 or KCNJ11 mutation just has the overexpression of paternally imprinted genes, while expression of the maternally imprinted genes such as KCNQ1 is decreased or lacking. **(D)** In Costello syndrome, the pathogenic HRAS mutation usually resides on the paternally inherited chromosome; other genes in the region are unaffected. **(E)** Somatic patUPD11p in Costello syndrome leads to the overexpression of paternally imprinted genes/lack of expression of maternally imprinted genes and also to a duplication of the mutated HRAS, which may contribute to dysregulation of proliferation/differentiation in affected cells. **(F)** Usher-CHI syndrome results from a homozygous contiguous gene deletion encompassing parts of ABCC8 and the USH1C genes.

side of hyperplasia). Mosaicism is particularly frequent in cases with patUPD11p, and a severe BWS phenotype is associated with high levels of somatic mosaicism for this anomaly (9). Familial occurrence of BWS is mostly associated with pathogenic variants in CDKN1C.

The incidence of neonatal hypoglycemia in BWS has been reported to be approximately 50% (10, 11). However, in most cases of BWS hypoglycemia is mild and transient. Notably, the onset of hypoglycemia can occasionally be delayed for several days, or even months (6). In BWS children with mild and transient hypoglycemia, the metabolic disturbance may resemble the one seen in newborns with fetal macrosomia due to maternal diabetes. However, in a minority of cases with BWS, hypoglycemia is more severe and persistent, showing the characteristics of CHI. Some patients are responsive to diazoxide (12) and some may even require pancreatic surgery (12–14). The overall prevalence of clinically significant and persistent CHI in BWS has not been determined, but is probably less than 10% (11). However, hyperinsulinism is one of the 6 cardinal features used in the clinical scoring system for the diagnosis of BWS (7).

The pathophysiology of CHI in BWS is not fully understood, but there is strong evidence for significant genotype-phenotype correlations. In a retrospective study on 28 children with CHI and a wide range of BWS-associated features ranging from classical BWS to oligosymptomatic cases with only mild hemihypertrophy, mosaic paternal uniparental isodisomy for chromosome 11p (patUPD11p) was found in 26 out of 28 cases (93%), while only two cases with CHI and BWS were associated with hypomethylation at IC2; these two had only mild CHI (14). This indicates that patUPD11p, which accounts for approximately 20% of cases with BWS in general, is the genotype that is specifically associated with a manifestation of CHI in BWS. This particular genotype-association is supported by other reports (13, 15–18) (Supplementary Table S1).

Four of the 28 cases reported by Kalish et al. (14) and a couple of other reported cases (15, 17) have been found to carry a paternally inherited K_{ATP} variant in addition to their patUPD11p, thus explaining the CHI phenotype on the same mechanistic basis as the established dual hit mechanism for focal CHI (Figure 1B). Not surprisingly, these patients had a much more severe course of HH than patients with patUPD11p alone (14). Pancreatic histology showed areas of irregular proliferation of endocrine tissue forming coalescing nodules and trabeculae with a more widespread pattern compared to typical focal pancreatic lesions (14, 15), a pattern that probably reflects the distribution of mosaicism for the patUPD11p in the pancreas. Therefore, the spectrum of disorders caused by the combination of a paternally inherited K_{ATP} channel mutation and a secondary somatic patUPD11p can be regarded as a continuum where the timing and distribution of the second event is critical: BWS symptoms represent the clinical correlate of an early embryonic occurrence

leading to systemic involvement with patUPD11p, while late emergence of patUPD11p that is restricted to the pancreas causes non-syndromic focal CHI (19).

However, the majority of cases with CHI and BWS due to patUPD11p reported by Kalish et al. were not associated with a K_{ATP} variant, suggesting that the UPD11p *per se* can also initiate CHI with a K_{ATP}-independent mechanism (14) (Figure 1C). The authors proposed a combined mechanism of expanded β-cell mass due to the pro-proliferative effect of the paternal imprint pattern at the 11p15 region together with functional abnormalities in β-cell insulin secretion possibly caused by the lack of the maternally expressed KCNQ1 voltage-gated potassium channel that is assumed to be involved in the regulation of potassium flux in pancreatic β-cells (14). Consistent with the assumption that patUPD11p in the pancreas can *per se* cause CHI, Flanagan et al. reported two cases of CHI with segmental patUPD11p in DNA extracted from pancreatic tissue and no K_{ATP} channel mutation (20). patUPD11p was not found in DNA from leukocytes and buccal cells and the patients were lacking clinical signs of BWS.

It has been reported that most of the patUPD11p patients without a paternally inherited K_{ATP} channel mutation do not require treatment beyond 2 years of age (12, 14). This might be related to the shift of KCNQ1 expression from monoallelic fetal to biallelic adult expression (21). Notably, in the subgroup of cases with patUPD11p retrieved by our literature search, persistent HI is more common, resistance to diazoxide prevails, and 17 of 48 cases with patUPD11p alone, as well as all cases with an additional K_{ATP} mutation received partial or subtotal pancreatectomies (Supplementary Table S1). A reporting bias towards more severely affected individuals and unidentified duplicates cannot be excluded.

Mosaic genome-wide paternal UPD is a very rare disorder with a predominant phenotype of patUPD11 (BWS-like) and a high risk of tumor development, which may by expanded by features of other patUPD-related disorders (22). CHI is a frequent complication (22, 23) and follows the same mechanism as in patUPD11p-related CHI discussed above. Similarly, a high rate of unresponsiveness to diazoxide and need for surgery was reported in published cases (Supplementary Table S1). **Multilocus imprinting disturbance** (MLID) is a primary epigenetic disorder where aberrant imprinting marks (most commonly loss of methylation) occur at multiple differentially methylated regions of the genome. It may be caused by defects in the subcortical maternal complex (SCMC) which is required for the proper oocyte maturation and early embryonic development (24). BWS-like features may be accompanied by symptoms of other imprinting disorders. Similar to BWS caused by epimutations, hypoglycemia may occur, but overt CHI is probably very rare.

3.2 Sotos syndrome

Sotos syndrome (OMIM #117550) is characterized by overgrowth (macrosomia and/or macrocephaly) with advanced bone maturation, developmental/neuropsychological deficits, and a distinctive facial appearance including a prominent forehead, dolichocephaly, sparse frontotemporal hair, downslanting palpebral fissures, malar flushing, and a long face. Affected individuals may also display cardiac or renal anomalies, musculoskeletal abnormalities, seizures, and a variety of other abnormalities. Sotos syndrome is estimated to occur in 1:14,000 live births (25). Sotos syndrome can be caused by a heterozygous pathogenic variant in NSD1 or a deletion encompassing NSD1. In the vast majority of cases the causative variant is *de novo* (25).

Neonatal hypoglycemia is a known feature of Sotos syndrome but it has been observed in less than 15% of affected individuals (25). However, several well-documented cases with Sotos syndrome and CHI have been reported (26–30). In the first reported cases, NSD1 microdeletions predominated, leading to the speculation that additional genes in the deleted 5q35 region might be critical for the metabolic abnormality (26, 27). However, subsequent reports on several patients with Sotos syndrome and CHI who carried intragenic NSD1 mutations rather suggest that the defect in NSD1 itself is sufficient to cause CHI (28–30).

The precise mechanism of dysregulated insulin secretion in Sotos syndrome is unknown. Notably, the gene product of NSD1 is a histone methyltransferase that is known to be involved in the regulation of chromatin and gene expression, and circumstantial evidence exists that NSD1 may thereby also regulate islet cell insulin expression (30).

In the majority of cases of Sotos syndrome CHI was transient, but Grand et al. also reported three patients with CHI persisting up to the age of 4 years (30). Responsiveness to diazoxide treatment was commonly observed (30), and no case of pancreatectomy has been reported (Supplementary Table S1).

3.3 Other overgrowth syndromes

For **Malan syndrome** (also known as Sotos syndrome 2, OMIM #614753), no cases of CHI have been reported to date (31).

Simpson-Golabi-Behmel syndrome (SGBS; OMIM #312870) is characterized by pre- and postnatal macrosomia, distinctive craniofacial features, intellectual disability with or without structural brain anomalies, and variable other anomalies. It is caused by pathogenic variants in GPC3 and inherited in an X-linked manner with possible disease manifestation also in females. Neonatal hypoglycemia was mentioned as a possible complication "as in other overgrowth syndromes" in a review on SGBS (32), but its frequency is unknown and detailed clinical reports documenting CHI are lacking. Interestingly, experimental studies have shown that adipocytes of SGBS patients are more sensitive to insulin stimulation, which may cause increased glucose uptake and thereby cause hypoglycemia (33).

Neonatal hypoglycemia has anecdotally been reported in **Weaver syndrome** (OMIM #277590), an overgrowth syndrome with variable intellectual disability and characteristic facial features, caused by heterozygous EZH2 pathogenic variants (34), but CHI has not been documented in the literature, so far.

Perlman syndrome (OMIM #267000) is an autosomal recessive condition caused by DIS3L2 mutations. Major clinical features neonatal macrosomia, facial anomalies, renal dysplasia,

nephroblastomatosis and multiple congenital anomalies. It is associated with high neonatal mortality (35). Neonatal hypoglycemia was repeatedly reported. Autopsy in one patient that died unexpectedly at 8 months of age revealed an increase in the number of the pancreatic islets, leading to the speculation that hyperinsulinism might play a role for fetal macrosomia and postnatal complications (36). In fact, CHI has not been documented in literature, to date.

Foster et al. reported infantile hypoglycemia in **Kosaki overgrowth syndrome** (OMIM #616592) caused by PDGFRB mutations (37). Tenorio et al. reported neonatal hypoglycemia in some of the patients affected by RNF125-related overgrowth syndrome (**Tenorio syndrome**; OMIM #616260) (38). In both cases there was not a diagnosis of hyperinsulinism. The wide range of prenatal overgrowth syndromes with a reported association with neonatal hypoglycemia suggests that a predisposition to disturbed neonatal glucose regulation might be a feature that is common to this group of disorders in more general.

3.4 PI3K-AKT pathway disorders

Human disorders caused by activating mutations in the PI3K-AKT pathway constitute a large group of diseases including Proteus syndrome (OMIM #176920), PIK3CA-related overgrowth spectrum (OMIM #612918), megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH; OMIM #PS603387) syndrome and others. Most of these entities are sporadic with mosaicism for the causative mutation, but in a few cases germline mutations have also been observed. The activating AKT2 p.Lys17Glu mutation (mosaic or germline) was identified in children with (asymmetric) overgrowth and severe recurrent hypoglycemia from infancy with a classical biochemical profile of hyperinsulinism (i.e., low serum levels of ketone and free fatty acids), but undetectable insulin (OMIM #240900) (39). Several other similar cases have been reported (40–42). AKT2 seems to be consistently associated with this metabolic phenotype. Further publications pointed out that hypoglycemia with a similar biochemical profile may also occur in a subset of patients with other PI3K-AKT pathway disorders caused by (mosaic) activating mutations in AKT3, PIK3CA, PIK3R2, and CCND2, thus suggesting that this type of metabolic dysregulation is in principle shared by the entire group of disorders (43, 44). Consistent with this, Saito et al. described a case of AKT1-caused Proteus syndrome with hypoinsulinemic hypoglycemia (45), and Liu et al. reported a case with PTEN-related overgrowth (OMIM #158350) and recurrent hypoketotic hypoglycemia (46). Onset of hypoglycemia in patients with PI3K-AKT pathway disorders was variable, mostly within the first years of life but not typically neonatal.

It is assumed that uncoupling of cellular responses to insulin that are mediated by the PI3K-AKT pathway, such as membrane translocation of the glucose transporter GLUT4, is the underlying mechanism (39). The hypoinsulinemic hypoglycemia of PI3K-AKT pathway disorders is therefore considered a mimicker or phenocopy of CHI (5). The clinical heterogeneity and apparent low penetrance of the metabolic phenotype (except for AKT2) may be explained by the mosaicism nature of these disorders. Leiter et al. speculated that more widespread distribution of mosaicism that involves also metabolic target organs like the liver is required to produce the hypoglycemia phenotype (43). A mouse model using inducible ubiquitous knock-in of the constitutively active $Pik3ca^{H1047R}$ mutation reproduced the human phenotype with overgrowth and metabolic abnormalities, including a reduction in blood glucose levels and undetectable insulin levels, thereby underscoring the critical role of PI3K in the regulation of glucose metabolism (47). Dushar et al. described response to sirolimus treatment in a family with familial hypoinsulinemic hypoglycemia caused by the recurrent AKT2 mutation (48).

3.5 Kabuki syndrome

Kabuki syndrome (OMIM #PS147920) is a developmental disorder characterized by recognizable facial features, intellectual disability, postnatal growth deficiency and variable congenital malformations. Pathogenic variants in the autosomal gene KMT2D or in the X-chromosomal gene KDM6A account for approximately 75% and 3-5% of cases, respectively (49). Both genes encode for histone-modifying enzymes, thus placing Kabuki syndrome in the group of disorders of chromatin regulation.

Neonatal hypoglycemia appears to be quite frequent in Kabuki syndrome and several cases of CHI have been reported. CHI may be the presenting feature of the disease (50). However, the prevalence figures in the literature are conflicting: In a congress report by de Leon et al. it was mentioned that in up to 70% of children with Kabuki syndrome CHI was observed (51), a prevalence figure that was cited by others, although no reference to original data has been provided. Genevieve et al. presented a series of 20 patients and reviewed 313 published cases with an overall prevalence of approximately 7% for neonatal hypoglycemia and 2% for persistent hypoglycemia and/or CHI (52). These figures are likely to be an underestimation, as it cannot be assumed that a proper assessment for neonatal hypoglycemia and CHI was conducted for each of these patients. CHI in Kabuki syndrome has also been described in a number of case reports (53–59). Yap et al. reported 10 cases of Kabuki syndrome with CHI/HH and suggested that the rate of hyperinsulinism among patients with Kabuki syndrome might be higher than previously assumed (50). In a series of 69 patients with syndromic HH, Kostopoulou et al. reported 9 cases of Kabuki syndrome (5), making it the second most common diagnosis in this cohort after BWS.

Notably, there is convincing evidence of significant genotype-phenotype correlations with a higher prevalence of neonatal hypoglycemia and hyperinsulinism in patients with KDM6A-caused Kabuki syndrome (50, 60, 61). Faundes et al. presented a large cohort of KDM6A-caused Kabuki syndrome and a review of the literature yielding a prevalence in the overall cohort of 56% for neonatal hypoglycemia and 28% for hyperinsulinism (61). This suggests that – although the predisposition to hyperinsulinism applies to Kabuki syndrome, in general – the defect of KDM6A may more specifically impact β-cell function compared to a defect of KMT2D (60). The precise mechanism for CHI in Kabuki

syndrome remains to be elucidated. Considering the molecular basis of this syndrome it is conceivable that epigenetic mechanisms in metabolic regulation are affected by the underlying defect in chromatin modification.

The majority of patients with Kabuki syndrome and CHI respond to diazoxide and hyperinsulinism typically resolves within the first two years of life (5, 51). However, few patients also underwent pancreatectomy (5) (Supplementary Table S1).

3.6 Costello syndrome

Costello syndrome (OMIM #218040) belongs to the RASopathies, a group of developmental syndromes caused by mutations in components or modulators of the RAS-MAPK pathway. Costello syndrome is characterized by congenital heart defects, myocardial hypertrophy, feeding difficulties, failure to thrive in infancy, postnatal growth delay, distinctive craniofacial features, developmental delay/intellectual disability, and tumor predisposition. Specific activating HRAS variants (most commonly p.Gly12Ser), which are also known as somatic oncogenic mutations, are causative for this disease (62). The HRAS gene is located on chromosome 11p15.5 close to the BWS region (Figure 1D).

Neonatal hypoglycemia is quite common in Costello syndrome, it has been reported with a frequency of 44% (63). CHI, however, has only occasionally been documented (64–66). Sheffield et al. reported one case with Costello syndrome and CHI where autopsy identified a pancreatic nodule with morphologic and immunohistochemistry findings similar to a focal lesion of CHI. No K_{ATP} channel mutation was detected (65). In another patient with severe neonatal hypoglycemia, Kerr et al. reported a "nesidioblastosis-like" lesion with islet hypertrophy and hyperplasia (67). Gripp et al. demonstrated patUPD11p in the focal lesion from the patient reported by Sheffield et al. (68), thus suggesting a similar pathophysiology for CHI as in BWS in this particular case (Figure 1D). Notably, patUPD11p is known as a somatic driver event in Costello syndrome-associated tumors (69). It remains questionable that all cases of CHI in Costello syndrome are accounted for by this mechanism. No such investigations have been reported in other patients. Given the frequency of neonatal hypoglycemia in Costello syndrome, it seems that intrinsic mechanisms driven by mutant HRAS itself are involved, which are not known in detail. Notably, metabolic disturbances with hypoglycemia and hypercholesterolemia have been recognized as a frequent finding in Costello syndrome beyond infancy (70). Oba et al. generated mice with the Costello syndrome-associated Hras mutation G12S and observed hypoketosis and elevated levels of long-chain fatty acylcarnitines under starvation conditions suggesting impaired mitochondrial fatty acid oxidation. They concluded that the mutant Hras modulates energy homeostasis *in vivo* (71).

Data on management and long-term outcome of CHI in Costello syndrome are scanty. Responsiveness to diazoxide has been reported; one patient underwent pancreatectomy (12) (Supplementary Table S1).

Neonatal hypoglycemia has also been reported in other RASopathies (63), but it occurs less frequently than in Costello syndrome (9% in Noonan and 6% of cardio-facio-cutaneous syndrome patients) and cases of documented CHI are lacking.

3.7 Turner syndrome

Turner syndrome is a relatively common sex chromosome abnormality affecting approximately 1 in 2,500 live female births. It is caused by monosomy X (karyotype: 45,X) or various other X-chromosomal abnormalities leading to partial monosomy. Short stature and primary amenorrhea due to ovarian dysgenesis are hallmarks of the disease. Several cases of CHI/HH in girls with Turner syndrome or mosaic Turner syndrome have been reported (72–74), suggesting an increased incidence of CHI/HH in this disorder, the precise dimension of which remained however undetermined. Gibson et al. presented 12 girls with Turner syndrome in combination with CHI (75). Based on their patient cohort, the authors estimated that the risk of CHI/HH in girls with Turner syndrome might be increased by about 50-fold compared to general population (75). Kostopoulou et al. identified 6 cases of Turner syndrome in their cohort of 69 patients with syndromic CHI/HH, making Turner syndrome the third most common syndromic cause of CHI/HH in this series (5).

The underlying mechanism leading to hyperinsulinism in Turner syndrome remains unclear. It has been speculated that the loss of one copy of the KDM6A gene which is located on Xp might play a role, thus suggesting a similar mechanism for CHI as in KDM6A-related Kabuki syndrome (75). This notion was supported by the finding that islets isolated from the pancreas of one Turner syndrome patient showed abnormal regulation of insulin secretion, with increased sensitivity to amino acids and elevated basal cytosolic calcium, a phenotype that could be partially reproduced in mouse islets exposed to a KDM6A inhibitor (75).

A majority of the reported cases with Turner syndrome and CHI were responsive to diazoxide and resolution of HH frequently occurred within the first years of life (5, 75) (Supplementary Table S1). Few patients underwent pancreatectomy with a histopathology consistent with diffuse hyperinsulinism (75). The susceptibility to abnormal glucose homeostasis in infancy may be related with the increased susceptibility to insulin resistance and β-cell dysfunction which is a well-known feature in adolescent and adult females with Turner syndrome (76, 77).

3.8 Chromosome 9p deletion syndrome

Variably sized monosomy of the short arm of chromosome 9 may result from isolated deletions or unbalanced translocations. Terminal 9p deletions cause complex syndromic conditions with multiple congenital anomalies and developmental delay/intellectual disability (OMIM: #158170). CHI has been observed in a number of cases with monosomy 9p: Banerjee et al. reported 12 cases with neonatal hypoglycemia, ten of them with biochemically confirmed hyperinsulinism, and reviewed three previously reported cases (78).

Kostopoulou et al. observed one case with a chromosome 9 deletion and chromosome 2 duplication (not further specified) among 69 patients with syndromic neonatal hypoglycemia, but CHI was not confirmed in that case (5). The prevalence of CHI in patients with monosomy 9p is unknown, but it has been recognized in less than 10% of cases reported in the literature and databases.

The precise molecular mechanism of the dysregulated insulin secretion associated with monosomy 9p remains unknown. A minimal deleted region was mapped to 7.2 Mb, encompassing 38 protein-coding genes. SMARCA2 and RFX3 were proposed as potential candidates for the hypoglycemia, but no experimental evidence has been provided (78).

The course of CHI in monosomy 9p may be transient or persistent; responsiveness to diazoxide with treatment up to the age of 6 has been reported (Supplementary Table S1).

3.9 Usher-CHI syndrome (homozygous 11p15-p14 deletion syndrome)

Usher-CHI syndrome (OMIM #606528) is the unique combination of two autosomal recessive syndromes caused by homozygosity for a recurrent 122 kb deletion which encompasses parts of the neighboring genes ABCC8 and USH1C, USH1C:c.(90 + 592)_ABCC8:c.(2694–528)del) (Figure 1F). The condition was first described by Bitner-Glindzicz et al. with the clinical features of CHI, congenital sensorineural deafness, developmental delay, enteropathy, and renal tubular dysfunction (79). Hussain et al. (80) and Al Mutair et al. (81) reported additional cases.

CHI in this condition is similar in its clinical presentation and pathophysiology to the ABCC8-deficient autosomal recessive diffuse form, while the remainder of the phenotype represents Usher syndrome type 1C and is explained by the USH1C defect. Accordingly, these patients do usually not respond to diazoxide, and most of the published cases underwent surgery (Supplementary Table S1).

3.10 Other chromosomal contiguous gene deletions reported as associated with CHI/HH

Trisomy 13 or mosaic trisomy 13 have been anecdotally reported in association with CHI (82–84). Shiu et al. added a case of CHI with partial trisomy 13: 47,XY,+del(13)(q14q32) (85). Kostopoulou observed another case in their series of 69 children with syndromic CHI (5). The potential molecular mechanism underlying CHI in trisomy is unclear and other genetic causes of CHI have not been excluded in those children.

Kostopoulou et al. reported two patients with CHI and a **16p11.2 microdeletion** (OMIM #613440) (86). Hoytema van Konijnenburg observed CHI in a patient with Zellweger syndrome who was found to have also a 16p11.2 microdeletion. Based on the lack of descriptions of CHI in Zellweger syndrome and the previous publication by Kostopoulou et al., the authors discussed that CHI in their patient was most likely caused by the 16p11.2 deletion syndrome (87). The 16p11.2 recurrent deletion phenotype includes a variable spectrum of developmental delay/intellectual disability, psychiatric conditions, autistic features, and epilepsy (88). Notably, early-onset obesity is a known feature of this disorder (88, 89) and may be associated with secondary hyperinsulinism (90). This might point towards a possible primary metabolic alteration in this syndrome. However, it has to be taken into account the prevalence of approximately 1:2000 live births in the general population for 16p11.2 microdeletions. Therefore, a chance association for the few reported cases with CHI cannot be excluded. Baple et al. et al. reported one case of syndromic CHI with a *de novo* interstitial **12q24.31 deletion**. The authors discussed that the CHI phenotype could be accounted for by haploinsufficiency of HNF1A which was contained in the deleted interval (91).

Kostopoulou reported one case of **trisomy 21** among 69 children with syndromic CHI (5). No such cases had been described before. However, more recently Hewat et al. reviewed cases referred for genetic testing of CHI in a national reference center. They identified 11 individuals with Down syndrome in a cohort of 2011 patients referred for genetic testing for CHI, which represents an increased prevalence compared to the general population (92). A pathogenic ABCC8 mutation was identified in one of the 11 individuals, probably explaining the CHI phenotype in this child. Five others were reported to have non-genetic risk factors for hyperinsulinism resulting from co-morbidities (intrauterine growth retardation, prematurity, gastrointestinal surgery possibly leading to dumping syndrome, L-asparaginase treatment). Similar results were reported in a retrospective study from Finland where five cases with Down syndrome were identified in a cohort of 238 individuals, one of them with a pathogenic heterozygous KCNJ1 variant (93). In eight of the cases reported in these two studies CHI was reported as persistent (including the two cases with K_{ATP} mutation) and in seven as transient. The majority of children requiring medical therapy responded to diazoxide; one received surgery (Supplementary Table S1). Hewat et al. concluded that the overrepresentation of Down syndrome in cohorts referred for CHI testing was likely due to an increased burden of non-genetic risk factors resulting from the Down syndrome phenotype (92).

The hypothesis raised by Hewat et al. regarding trisomy 21 (92) may also apply for other chromosomal disorders that have anecdotally been associated with CHI, including trisomy 13, which is discussed above. Kostopoulou et al. also reported one case of monosomy 22q11.2 (DiGeorge syndrome), as well as eight cases with other chromosomal anomalies (duplications/deletions) in their series of 69 cases with syndromic CHI (5). Giri et al. reported a patient with CHI and Poland syndrome, who had a 10p13–14 duplication (94).

3.11 CDG syndromes

Congenital disorders of glycosylation (CDG; OMIM #PS212065) constitute a large heterogeneous group of rare genetic disorders of glycan synthetic pathways with mainly autosomal recessive inheritance. They have a wide phenotypic spectrum with

multisystem involvement including failure to thrive, developmental delay, neurologic and ocular abnormalities, hepatopathy, enteropathy and others (95). The genes causing CDG syndromes encode enzymes of the glycan synthetic or interacting pathways. The estimated prevalence of CDG syndromes in Europe is 1:22,000 live births; the most common defect is PMM2-CDG, followed by ALG6-CDG, ALG1-CDG, and MPI-CDG (96).

Recurrent hypoglycemia is a known feature in CGD syndromes, and several cases have been described with neonatal or infantile HH. The entities reported to be associated with hyperinsulinism are PMM2-CDG (CDG1a), MPI-CDG (CDG1b), ALG3-CDG (CDG1d), and PGM1-CDG (CDG1t) (reviewed by 97, 98). This distribution seems to be not significantly different from the general prevalence of CDG subtypes, suggesting that disturbed blood glucose regulation is rather a disease group feature than specific for certain entities. However, it can be noted that HH seems to be particularly common in MPI-CDG where hypoglycemia was reported in a majority of patients with a mean age of presentation of 6.8 months and hyperinsulinism in two thirds of hypoglycemic patients (99). CHI may be the presenting sign of MPI-CDG (100, 101). Well-documented cases of hypoglycemia in CDG syndromes are otherwise rare. For the most common type, PMM2-CDG (CDG1a), Vuralli counted 37 affected children among 1060 published cases (3,4%) (98). Manifestation of hypoglycemia was mostly in the first months of life and in six of the reported cases it was the first presenting symptom. Hyperinsulinism was confirmed in about half of the cases (10/22) from which appropriate clinical and laboratory data were published, and the majority of them responded to diazoxide. The authors presented three new cases and proposed that hyperinsulinism might be more frequent in PMM2-CDG than previously reported (98). Wong et al. observed hypoglycemia in 89% of patients with PGM1-CDG (CDG1t), but hypoglycemia at any age was included and hyperinsulinism was not reported (102). Sun et al. reported on case of ALG3-CDG (CDG1d) with CHI and islet cell hyperplasia on autopsy (103). HH in CDG syndromes is mostly responsive to diazoxide. Oral mannose treatment has been reported to have a favorable effect on hypoglycemia in patients with MPI-CDG (99, 100).

The mechanism underlying hypoglycemia in CGG syndrome is unclear. A complex pathogenesis may be assumed, since these patients have multisystem involvement often with other endocrine, hepatic and other organ involvement. However, there is evidence that protein glycosylation may also be directly involved in glucose homeostasis. It has been demonstrated, for example, that SUR1 glycosylation is critical for the proper trafficking and surface expression of K_{ATP} channels (104).

Notably, a distinct promoter mutation (c.-167G>T) in the PMM2 gene, either homozygous or in trans with other PMM2 coding mutations, was identified in several unrelated individuals with a phenotype of HH and congenital polycystic kidney disease, who did not exhibit the typical clinical or diagnostic features of CDG1a (105). The diagnosis of HH was within the first year of life in 11 out of 17 children and in the newborn period in four. The authors proposed that the PMM2 promoter mutation might alter tissue-specific chromatin loop formation, with consequent organ-specific deficiency of PMM2 explaining the restricted phenotype. Soares et al. presented another case of CHI and polycystic kidneys with that particular PMM2 variant (106). Chen et al. recently reviewed this particular clinical and genetic subtype of CDG syndromes under the term PMM2-HI (107).

CHI associated with CDG syndromes has commonly been reported to respond to diazoxide, while our literature search revealed only one case (1 of 38) treated with pancreatic surgery (Supplementary Table S1).

3.12 Other monogenic syndromic conditions reported as associated with CHI/HH

Heterozygous mutations of **CACNA1C**, which is expressed in the Cav1.2 (α1C-containing) Ca2+ channels can cause a variety of disorders with cardiac arrhythmias as the leading symptom (Timothy syndrome, Brugada syndrome, Long-QT syndrome) with distinct genotype-phenotype correlations. Timothy syndrome (OMIM #601005) is a complex syndromic condition with a combination of prolonged QT interval, congenital heart defects, syndactyly, facial anomalies, and neurodevelopmental delay. Most patients share the same pathogenic variant (p.Gly406Arg), and a similar phenotype but without syndactyly is associated with similar but distinct pathogenic variants (108). Intermittent hypoglycemia has been observed in approximately 40% of patients with Timothy syndrome and was speculated to be accounted for by episodic dysfunction of Cav1.2 (109, 110). Only one published case with CACNA1C-related disease and confirmed CHI was retrieved by our literature search (111).

De novo heterozygous **CACNA1D** missense mutations have been described in two patients with CHI, cardiovascular anomalies and neurodevelopmental problems (112, 113). One of them had also primary hyperaldosteronism (113). CACNA1D mutations have previously been associated with primary aldosteronism, seizures, and neurologic abnormalities (PASNA; OMIM #615474), thus suggesting a disease spectrum that may include CHI with reduced penetrance. CACNA1D gene encodes one of several α1 subunits of L-type voltage-gated calcium channel. These channels are widely expressed in mammalian organs including pancreatic islets (114). Cav1.3 (α1D-containing), as well as Cav1.2 (α1C-containing) Ca2+ channels have differential modulatory effects on glucose-stimulated insulin secretion (115, 116).

KCNQ1 is a gene located in the 11p15.5 differentially methylated region (Figure 1A) and encodes a potassium channel. A possible role of reduced expression of KCNQ1 in BWS-associated CHI has been discussed above. KCNQ1 causes Long-QT syndrome (OMIM #192500) and other types of cardiac arrhythmias. Torekov et al. pointed out that patients with KCNQ1-related Long-QT syndrome may exhibit hyperinsulinemia and symptomatic reactive hypoglycemia after glucose challenge (117). Experimental data on Kcnq1-mutant mice showing age–dependent transition from islet insulin hypersecretion to hyposecretion support the role of this potassium channel in insulin regulation (118). Similar findings as in KCNQ1-related Long-QT syndrome were made in patients with mutations in KCNH2, the second most common

cause of Long-QT syndrome (OMIM #613688) (119). No well-documented reports on CHI with either KCNQ1 or KCNH2 mutations exist in the literature. In summary, it seems to be plausible that potassium channel mutations other than K_{ATP} may have impact on insulin regulation in pancreatic β-cells, but their relevance for CHI remains unclear.

FOXA2-CHI: Giri et al. identified a *de novo* heterozygous mutation in FOXA2 (c.505T>C, p.S169P) in a child with CHI and congenital hypopituitarism, craniofacial anomalies, choroidal coloboma, cardiovascular and malformations gastrointestinal abnormalities, and developmental delay (120). Additional single case reports described patients with hypopituitarism and HH, and variable other abnormalities, who carried *de novo* FOXA2 mutations (121, 122). FOXA2 point mutations and deletions have also been reported patients with syndromic hypopituitarism but without documented hyperinsulinism (reviewed by 124). Current evidence thus suggests that FOXA2 should be considered in the differential diagnosis of HH especially when pituitary deficiencies co-exist (95). FOXA2, also known as HNF3B, is conserved transcription factor that is involved in the development of endoderm-derived organs including the pancreas (123) and acts as an activator of genes that function in multiple pathways governing insulin secretion (121, 124). FOXA2 has also been proposed to act as a metabolic sensor in hypothalamic neurons (125), and its role in glucose metabolism is supported by the finding that tissue-specific deletion of Foxa2 in pancreatic β-cells in mice results in HH (126). We retrieved four cases with FOXA2 mutations and a confirmed diagnosis of CHI in the literature; two out of three receiving treatment with diazoxide were described as (partial responders (Supplementary Table S1).

Three males from one family with a variant in **EIF2S3** were reported with an unusual dysregulation of glucose fluctuating between diazoxide-responsive HH and postprandial hyperglycemia diagnosed in childhood, along with learning difficulties and hypopituitarism (127). EIF2S3 encoding a subunit of the eukaryotic translation initiation factor 2, eIF2γ, and hemizygous mutations have been associated with a more severe syndrome of developmental delay/intellectual disability, epilepsy, hypogonadism, microcephaly, and obesity (MEHMO syndrome, OMIM #300148). Neonatal hypoglycemia as well as early-onset diabetes have been observed in patients with MEHMO syndrome (128). EIF2S3-mutated individuals display a complex metabolic-endocrine phenotype that may initially resemble CHI (129).

Congenital central hypoventilation syndrome (CCHS; OMIM #209880) may be associated with episodic hypoglycemia, sometimes manifesting with hypoglycemic seizures (130). Hyperinsulinemia has been reported in several cases (131–134), but in only few of them the manifestation was within the first weeks of life (131, 133). Most of the patients had a typical polyalanine expansion mutation in the PHOX2B gene as usually found in CCHS without hyperinsulinism. CCHS is a disorder of autonomic dysfunction and it has been speculated that this might also explain the predisposition to disturbance of glucose homeostasis (130, 134). However, manifestation of CHI appears to be very rare in CCHS. When necessary, conventional pharmacological treatment was found efficient (Supplementary Table S1).

HH has been described in a small number of patients with **Rubinstein-Taybi syndrome** (RTS; OMIM # PS180849**)** (5, 135–138). Age at diagnosis of HH was variable; only few cases had well-documented neonatal onset justifying the diagnosis of CHI (135, 137). The prevalence of CHI/HH in RTS is not known, but seems to be quite low (less than 5%). A recent meta-analysis of EP300-mutated RTS identified hypoglycemia in three subjects with documented neonatal onset in two of them (139). A majority of cases with RTS and HH that have a reported genotype had EP300 mutations, which only account for about 10% of RSTS patients, in general. Kostopoulou et al. reported two cases of RTS without genotype information in a cohort of 69 patients with syndromic CHI (5). The EP300 and CREBBP genes encode p300 and CBP, respectively, which function as transcriptional coactivators with histone acetyltransferase activity (histone modification) and are – among various other functions – also involved in islet cell development (140).

Sekiguchi et al. reported a case of **CHARGE syndrome** (OMIM #214800) caused by a CHD7 point mutation with HH in infancy (141). Kostopoulou et al. reported another case (5). CHD7 encodes a chromodomain helicase DNA-binding protein involved in chromatin remodeling and transcriptional regulation. Given the estimated prevalence of approximately 1:10,000 live births (142), the occurrence of HH in CHARGE syndrome can currently not be distinguished from a chance association.

Imaizumi et al. reported a case of **Coffin-Siris syndrome** (OMIM # PS156200) presenting at 4 month of age with recurrent hypoglycemia attacks (143). Kostopoulou et al. reported another case with HH (5). Additional cases of hyperinsulinism in Coffin-Siris syndrome appear in the literature, but they are not congenital but rather obesity-associated (144, 145). Coffin-Siris syndrome is a heterogeneous disorder of the SWI/SNF chromatin remodeling complex. Current evidence for a significant role in syndromic CHI is limited.

A syndromic condition comprising CHI, renal tubular dysfunction (Fanconi syndrome) and transient or recurrent hepatic dysfunction (OMIM #616026) was reported in patients with a heterozygous mutation of **HNF4A**, a gene that is known to be associated with MODY1 (OMIM #125850), but initial presentation in infancy may be CHI (146–148): Flanagan 2010, Stanescu 2012, Hamilton 2014). Notably, the syndromic condition including CHI, renal and hepatic disease has exclusively been reported in patients with the same HNF4A missense variant, p.Arg63Trp (R63W, also known as R76W or R85W). More than 20 cases have been reported (Supplementary Table S1). All patients presented with renal Fanconi syndrome and showed transient CHI. Later development of MODY was reported in some. About half of them developed recurrent benign hepatic dysfunction (reviewed by 149).

Soden et al. reported siblings with a heterozygous pathogenic MAGEL2 variant (**Schaaf-Yang syndrome**; OMIM #615547), probably based on parental germ cell mosaicism, who presented with CHI (150). Another case was reported by Halloun et al. (151). Neonatal hypoglycemia was also reported in further cases with this condition but were mostly attributable to growth hormone deficiency or adrenal insufficiency, or the etiology of the hypoglycemia has not been determined (151).

In a consanguineous family with microcephaly, short stature, and hyperinsulinemic hypoglycemia, Gillis et al. identified homozygosity for the missense variant G206R in the **TRMT10A** gene. Manifestation of hypoglycemic seizures in three affected siblings was between age 5 and 9 (152). A homozygous mutation in this gene was previously reported in another consanguineous family with a syndrome of young onset diabetes, short stature and microcephaly with intellectual disability (153). Hyperinsulinemic hypoglycemia was observed in a 3 months-old individual with a homozygous **YARS** mutation (encoding a tRNA synthetase) (154). Other cases with this disorder displaying infantile hypoketotic hypoglycemia have been described (155). The validity of the association with CHI in these extremely rare recessive diseases remains to be confirmed by additional observations. Kostopoulou et al. also reported one case of Alagille syndrome and one with Prader-Willi syndrome in their series of 69 cases with syndromic CHI (12), but no details were provided leaving the association of CHI with these disorders uncertain.

Tyrosinemia type 1 (OMIM #276700) is a rare metabolic disorder caused by a defect of fumarylacetoacetate hydrolase (encoded by FAH gene). It typically manifests in young infants with liver dysfunction and renal tubular dysfunction. Affected children may occasionally present with CHI (156). Sethuram et al. observed CHI also in one case of transient tyrosinemia of the newborn, which is a benign condition with a maturational defect of the enzymes associated with tyrosine metabolism without any genetic abnormalities (157). CHI in tyrosinemia type 1 is responsive to diazoxide and usually resolves within the first years of life (156, 157). Although islet-cell hypertrophy and hyperplasia have been reported in a number of cases of tyrosinemia type 1 (158), the precise pathophysiology of hyperinsulinism remains obscure. Hyperinsulinemic hypoglycemia may also be a presenting sign in other inherited metabolic diseases that may manifest with a complex syndromic phenotype, such as **adenosine kinase deficiency** (OMIM #614300) (159).

4 Discussion

The chromosomal region 11p15 plays a key role in CHI, as it contains the genes for the two components of the islet cell-specific K_{ATP} channel in 11p15.1. The 11p15 region is also involved in several syndromic forms of CHI (Figure 1), first and foremost BWS, which represents the most common form of syndromic CHI (5). However, the mechanisms of abnormal glucose regulation in 11p15-related disorders are variable and are not directly linked to defective K_{ATP} channel function in all of them (e.g. most cases with Beckwith-Wiedemann syndrome, Costello syndrome). Dysregulated expression of genes of the differentially methylated region in 11p15.5 is likely to play an important role, but their impact on metabolic programming the disturbance of which can lead to CHI is incompletely understood.

The broad and heterogeneous spectrum of syndromic disorders having reported associations with CHI/HH is intriguing. A few disease groups stand out from this diverse mixture and point at shared pathophysiologies: Overgrowth syndromes (Beckwith-Wiedemann syndrome, Sotos syndrome and others) seem to convey particular susceptibility to neonatal and infantile hypoglycemia with or without proven CHI. The abnormal metabolic and growth regulation that leads to intrauterine overgrowth may at the same time predispose to hypoglycemia and inappropriate insulin secretion in early postnatal life. Thorough investigation of patients presenting with neonatal hypoglycemia may in future reveal cases with CHI-like patterns in prenatal overgrowth conditions where CHI has not been documented, so far. This notion is supported by the observation of neonatal hypoglycemia in several rare overgrowth syndromes, such as Simpson-Golabi-Behmel, Weaver, Perlman, Kosaki and Tenorio syndromes (32, 34–38). For channelopathies that are associated with syndromic forms of CHI the shared mechanism leading to hyperinsulinism is probably their role in the regulation of ion currents in pancreatic β-cells, while additional manifestations reflect the function in other organs (e.g. the myocardium). Syndromic disorders associated with impaired glucose metabolism and early-onset diabetes may manifest with CHI in early infancy as a common pattern (HNF4A, HNF1A microdeletion, EIF2S3). Transient HI followed by impaired glucose tolerance and diabetes was also described in FOXA2-related CHI (122).

It is notable that among the disorders where CHI has been observed in a syndromic context, there are a number of variable chromosomal aneuploidies as well as several disorders of chromatin regulation. These may have in common the disturbance of the fine-tuning of gene expression, which does also play an important role in the fetal and neonatal metabolic programming. The precise mechanisms of CHI in these conditions remain obscure and are probably complex. If the hypothesis is true that a broad range of disturbances in the fine regulation of gene expression and cellular programming may affect the delicate shift in metabolic adaptation at the transit from intrauterine to postnatal life, it can be expected that cases of CHI/HH will also be occasionally observed in a variety of other syndromic disorders, in future. Such a hypothesis of metabolic maladaption would be consistent with the observation that CHI/HH was mostly transient in those disorders. On the other hand, it has to be considered that increased risk of CHI/HH in such complex disorders may also be due to an increased burden of non-genetic risk factors resulting from the underlying disease, such as intrauterine growth restriction, prematurity, and gastrointestinal or cardiac malformations (91).

It has to be pointed out that for many of the conditions discussed in this review – particularly in the group of chromosomal and monogenic developmental syndromes – CHI has only been documented in a small number of cases. Even for syndromes with a well-established association to CHI, the rate of affected individuals hardly reaches 10%. For those conditions, where only a few or single anecdotal reports exist, chance associations cannot be excluded and reporting of additional cases as well as experimental studies are necessary to further corroborate the causal link. However, it is also possible that CHI is under-diagnosed especially in disorders that present with complex medical issues, and a detailed metabolic and endocrine workup may be omitted or not reported especially in cases where the CHI features

are transient, which is often the case in syndromic types. The paucity of observations of CHI in conditions for which hundreds of reported cases exist in the literature argues in favor of a complex pathophysiology in which the underlying genetic condition plays a role as predisposing factor but is alone insufficient to produce the CHI/HH phenotype.

Susceptibility to (neonatal) hypoglycemia appears to be a shared feature of the various entities within certain pathophysiologically related disease groups (e.g. RASopathies, chromatin disorders, CDG syndromes), while CHI has only been documented in distinct entities out of those groups. This suggests that the common pathogenetic mechanism within certain disease groups generally predisposes to the metabolic dysregulation but with variable severity and penetrance. In cases where neonatal hypoglycemia is only mild and transient, hyperinsulinism is likely to remain undiagnosed. In fact, many publications reporting neonatal hypoglycemia in the disorders discussed in this review, do not report detailed metabolic investigations. The metabolic dysregulation as a disease-group phenomenon with variable penetrance and expression in the different entities/genotypes also seems to apply for the PI3K-AKT pathway disorders, where a distinctive mechanism of uncoupling of cellular metabolic response from insulin leads to the specific non-hyperinsulinemic CHI phenocopy.

In most of the CHI-associated syndromic conditions, the precise mechanism of dysregulation of glucose-sensing and/or insulin secretion is not completely understood and not directly related to known genes involved in isolated CHI. As mentioned above, in many of them the association with CHI is inconsistent and the metabolic disturbance is transient. However, since neonatal hypoglycemia is an early sign of possible compromise in the newborn, which requires immediate diagnostic efforts and intervention, this symptom may be the first to bring a patient to medical attention. Untreated hypoglycemia poses individuals affected by CHI at risk for central nervous system complications (1). Thereby, the consequences of CHI can add to neurodevelopmental deficits in syndromic disorders, the contribution of which may be difficult to delineate.

As mentioned above, diagnosis of a primary disorder of glucose regulation may be delayed or even be missed in complex clinical szenarios, when an infant has multiple clinical issues and medical interventions. Observations of neonatal hypoglycemia as a recurrent feature in some of the syndromic conditions discussed in this review with scarce documented cases of CHI may be taken as an indication of possible missed diagnoses of transient hyperinsulinemia in those disorders. As a consequence, it has been recommended that recurrent hypoglycemia should be assessed thoroughly in children with a syndromic clinical presentation. And children with features suggestive of syndromes associated with CHI/HH must be closely monitored for hypoglycemia and, when detected, be screened for possible hyperinsulinism (5). Identifying the genetic cause of CHI in a newborn with associated congenital anomalies or additional medical issues remains a differential diagnostic challenge and may require a broad genetic workup (5, 12, 160, 161). Depending on the clinical presentation, this should include testing for BWS, particularly patUPD11p, microarray analysis to detect chromosomal aneuploidies, as well as the analysis of a number of genes by multi-gene panel, exome or genome sequencing. In a recent review, Hewat et al. pointed out the usefulness of a comprehensive screening using targeted gene panels, exome, or genome sequencing for genetic testing for CHI, but it should be recognized that limitations remain with next-generation sequencing, and additional investigations (e.g. for the detection of copy number changes and methylation defects) may be required (162). It should also be noted that the epigenetic or genomic causes for BWS, especially patUPD11p, may not be detectable in leukocyte DNA and may require other DNA sources for detection (buccal cells, fibroblasts) (6).

Identification of the underlying cause of a syndromic disease with CHI may also have impact on individual surveillance and personalized treatment. A diagnosis of BWS should lead to the recommended tumor surveillance (6). Knowing the underlying genetic condition may also help to better assess the prospects of success of diazoxide treatment or pancreatic surgery. BWS due to patUPD11p, mosaic genome-wide patUPD and Usher-CHI syndrome have the highest rates of non-responsiveness to diazoxide (50% or more) and required pancreatectomies in one third or more of the reported cases. In contrast, the other syndromic types of CHI were mostly described to respond to conventional treatment with diazoxide and/or octreotide and surgery was performed only occasionally (Table 1 and Supplementary Table S1). Response to Ca2+ channel blockers has been reported for CHI caused by mutated CACNA1D (109) and a favorable effect of oral mannose treatment on HH has been observed in MPI-CDG (97, 98). More personalized therapies for rare diseases are likely to emerge in the future.

Finally, genetic counseling should generally be offered to parents of a child diagnosed with a syndromic disorder. Many of the diseases reviewed here are due to *de novo* dominant mutations and have a low risk of recurrence in the affected family (Table 1). However, the rare possibility of parental germ cell mosaicism cannot be excluded, and recurrence of CHI in siblings has, for example, been reported in Schaaf-Yang syndrome (150). Autosomal recessive or X-linked inheritance (Table 1), as well as familial balanced chromosomal translocations as reported, for example, in one family with 9p monosomy (78) may be associated with a substantial risk of recurrence. Since several of those syndromes have serious consequences on health and life quality besides the ones conferred by CHI itself, prenatal counseling and genetic testing may be indicated.

5 Conclusions

Syndromic disorders that have been found to be associated with CHI/HH comprise a very heterogeneous spectrum of diseases. For several of them the association is only supported by a few observations, but undiagnosed cases are likely to exist particularly in conditions where CHI is only transient. The pathophysiology underlying CHI remains obscure for many of these disorders, and the wide spectrum of syndromes with very different genetic causes suggest that the list of syndromes with occasional manifestation of

CHI will further increase. A broad genetic workup is recommended for newborns or infants presenting with CHI/HH and associated congenital anomalies or additional medical issues.

Author contributions

MZ contributed to the conception and the writing of the article. KP gave the constructive discussions to the article. KM revised important intellectual content critically for important intellectual content. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1013874/full#supplementary-material

References

1. Banerjee I, Raskin J, Arnoux JB, De Leon DD, Weinzimer SA, Hammer M, et al. Congenital hyperinsulinism in infancy and childhood: Challenges, unmet needs and the perspective of patients and families. *Orphanet J Rare Dis* (2022) 17(1):61. doi: 10.1186/s13023-022-02214-y

2. Finegold DN, Stanley CA, Baker L. Glycemic response to glucagon during fasting hypoglycemia: An aid in the diagnosis of hyperinsulinism. *J Pediatr* (1980) 96(2):257–9. doi: 10.1016/s0022-3476(80)80817-1

3. Hill DE. Fetal effects of insulin. *Obstet Gynecol Annu* (1982) 11:133–49.

4. Verkarre V, Fournet JC, de Lonlay P, Gross-Morand MS, Devillers M, Rahier J, et al. Junien C.Paternal mutation of the sulfonylurea receptor (SUR1) gene and maternal loss of 11p15 imprinted genes lead to persistent hyperinsulinism in focal adenomatous hyperplasia. *J Clin Invest* (1998) 102(7):1286–91. doi: 10.1172/JCI4495

5. Rosenfeld E, Ganguly A. De Leon DD.Congenital hyperinsulinism disorders: Genetic and clinical characteristics. *Am J Med Genet C Semin Med Genet* (2019) 181 (4):682–92. doi: 10.1002/ajmg.c.31737

6. Shuman C, Beckwith JB, Weksberg R. Beckwith-wiedemann syndrome. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2000).

7. Brioude F, Kalish JM, Mussa A, Foster AC, Bliek J, Ferrero GB, et al. Expert consensus document: Clinical and molecular diagnosis, screening and management of beckwith-wiedemann syndrome: an international consensus statement. *Nat Rev Endocrinol* (2018) 14(4):229–49. doi: 10.1038/nrendo.2017.166

8. Mussa A, Russo S, De Crescenzo A, Chiesa N, Molinatto C, Selicorni A, et al. Prevalence of beckwith-wiedemann syndrome in north West of Italy. *Am J Med Genet A* (2013) 161A(10):2481–6. doi: 10.1002/ajmg.a.36080

9. Smith AC, Shuman C, Chitayat D, Steele L, Ray PN, Bourgeois J, et al. Severe presentation of beckwith-wiedemann syndrome associated with high levels of constitutional paternal uniparental disomy for chromosome 11p15. *Am J Med Genet A* (2007) 143A(24):3010–5. doi: 10.1002/ajmg.a.32030

10. Mussa A, Di Candia S, Russo S, Catania S, De Pellegrin M, Di Luzio L, et al. Recommendations of the scientific committee of the Italian beckwith-wiedemann syndrome association on the diagnosis, management and follow-up of the syndrome. *Eur J Med Genet* (2016) 59(1):52–64. doi: 10.1016/j.ejmg.2015.11.008

11. DeBaun MR, King AA, White N. Hypoglycemia in beckwith-wiedemann syndrome. *Semin Perinatol* (2000) 24(2):164–71. doi: 10.1053/sp.2000.6366

12. Kostopoulou E, Dastamani A, Güemes M, Clement E, Caiulo S, Shanmugananda P, et al. Syndromic forms of hyperinsulinaemic hypoglycaemia-a 15-year follow-up study. *Clin Endocrinol (Oxf)* (2021) 94(3):399–412. doi: 10.1111/cen.14393

13. Laje P, Palladino AA, Bhatti TR, States LJ, Stanley CA, Adzick NS. Pancreatic surgery in infants with beckwith-wiedemann syndrome and hyperinsulinism. *J Pediatr Surg* (2013) 48(12):2511–6. doi: 10.1016/j.jpedsurg.2013.05.016

14. Kalish JM, Boodhansingh KE, Bhatti TR, Ganguly A, Conlin LK, Becker SA, et al. Congenital hyperinsulinism in children with paternal 11p uniparental isodisomy and beckwith-wiedemann syndrome. *J Med Genet* (2016) 53(1):53–61. doi: 10.1136/jmedgenet-2015-103394

15. Hussain K, Cosgrove KE, Shepherd RM, Luharia A, Smith VV, Kassem S, et al. Hyperinsulinemic hypoglycemia in beckwith-wiedemann syndrome due to defects in the function of pancreatic beta-cell adenosine triphosphate-sensitive potassium channels. *J Clin Endocrinol Metab* (2005) 90(7):4376–82. doi: 10.1210/jc.2005-0158

16. Adachi H, Takahashi I, Higashimoto K, Tsuchida S, Noguchi A, Tamura H, et al. Congenital hyperinsulinism in an infant with paternal uniparental disomy on chromosome 11p15: Few clinical features suggestive of beckwith-wiedemann syndrome. *Endocr J* (2013) 60(4):403–8. doi: 10.1507/endocrj.ej12-0242

17. Zarate YA, Shur N, Robin A, Garnica AD, Quintos JB, Schaefer GB. Persistent congenital hyperinsulinism in two patients with beckwith-wiedemann syndrome due to mosaic uniparental disomy 11p. *J Pediatr Endocrinol Metab* (2014) 27(9-10):951–5. doi: 10.1515/jpem-2013-0484

18. Kocaay P, Şiklar Z, Ellard S, Yagmurlu A, Çamtosun E, Erden E, et al. Coexistence of mosaic uniparental isodisomy and a KCNJ11 mutation presenting as diffuse congenital hyperinsulinism and hemihypertrophy. *Horm Res Paediatr* (2016) 85 (6):421–5. doi: 10.1159/000446153

19. Vossschulte H, Mohnike K, Mohnike K, Warncke K, Akcay A, Zenker M, et al. Correlation of PET-MRI, pathology, LOH, and surgical success in a case of CHI with atypical Large pancreatic focus. *J Endocr Soc* (2022) 6(6):bvac056. doi: 10.1210/jendso/bvac056

20. Flanagan SE, Kapoor RR, Smith VV, Hussain K, Ellard S. Paternal uniparental isodisomy of chromosome 11p15.5 within the pancreas causes isolated hyperinsulinemic hypoglycemia. *Front Endocrinol (Lausanne)* (2011) 2:66. doi: 10.3389/fendo.2011.00066

21. Travers ME, Mackay DJ, Dekker Nitert M, Morris AP, Lindgren CM, Berry A, et al. Insights into the molecular mechanism for type 2 diabetes susceptibility at the KCNQ1 locus from temporal changes in imprinting status in human islets. *Diabetes* (2013) 62(3):987–92. doi: 10.2337/db12-0819

22. Kalish JM, Conlin LK, Bhatti TR, Dubbs HA, Harris MC, Izumi K, et al. Clinical features of three girls with mosaic genome-wide paternal uniparental isodisomy. *Am J Med Genet A* (2013) 161A(8):1929–39. doi: 10.1002/ajmg.a.36045

23. Christesen HT, Christensen LG, Löfgren ÅM, Brøndum-Nielsen K, Svensson J, Brusgaard K, et al. Tissue variations of mosaic genome-wide paternal uniparental disomy and phenotype of multi-syndromal congenital hyperinsulinism. *Eur J Med Genet* (2020) 63(1):103632. doi: 10.1016/j.ejmg.2019.02.004

24. Eggermann T, Yapici E, Bliek J, Pereda A, Begemann M, Russo S, et al. Trans-acting genetic variants causing multilocus imprinting disturbance (MLID): Common mechanisms and consequences. *Clin Epigenet* (2022) 14(1):41. doi: 10.1186/s13148-022-01259-x

25. Tatton-Brown K, Cole TRP, Rahman N. Sotos syndrome. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2004).

26. Matsuo T, Ihara K, Ochiai M, Kinjo T, Yoshikawa Y, Kojima-Ishii K, et al. Hyperinsulinemic hypoglycemia of infancy in sotos syndrome. *Am J Med Genet A* (2013) 161A(1):34–7. doi: 10.1002/ajmg.a.35657

27. Nakamura Y, Takagi M, Yoshihashi H, Miura M, Narumi S, Hasegawa T, et al. A case with neonatal hyperinsulinemic hypoglycemia: It is a characteristic complication of sotos syndrome. *Am J Med Genet A* (2015) 167A(5):1171–4. doi: 10.1002/ajmg.a.36996

28. Carrasco Salas P, Palma Milla C, Lezana Rosales JM, Benito C, Franco Freire S, López Siles J. Hyperinsulinemic hypoglycemia in a patient with an intragenic NSD1 mutation. *Am J Med Genet A* (2016) 170A(2):544–6. doi: 10.1002/ajmg.a.37440

29. Cerbone M, Clement E, McClatchey M, Dobbin J, Gilbert C, Keane M, et al. Sotos syndrome presenting with neonatal hyperinsulinaemic hypoglycaemia, extensive thrombosis, and multisystem involvement. *Horm Res Paediatr* (2019) 92(1):64–70. doi: 10.1159/000496545

30. Grand K, Gonzalez-Gandolfi C, Ackermann AM, Aljeaid D, Bedoukian E, Bird LM, et al. Hyperinsulinemic hypoglycemia in seven patients with *de novo* NSD1 mutations. *Am J Med Genet A* (2019) 179(4):542–51. doi: 10.1002/ajmg.a.61062

31. Priolo M, Schanze D, Tatton-Brown K, Mulder PA, Tenorio J, Kooblall K, et al. Further delineation of malan syndrome. *Hum Mutat* (2018) 39(9):1226–37. doi: 10.1002/humu.23563

32. Sajorda BJ, Gonzalez-Gandolfi CX, Hathaway ER, Kalish JM. Simpson-Golabi-Behmel syndrome type 1. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2006).

33. Yeo CR, Agrawal M, Hoon S, Shabbir A, Shrivastava MK, Huang S, et al. SGBS cells as a model of human adipocyte browning: A comprehensive comparative study with primary human white subcutaneous adipocytes. *Sci Rep* (2017) 7(1):4031. doi: 10.1038/s41598-017-04369-2

34. Tatton-Brown K, Rahman N. EZH2-related overgrowth. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2013).

35. Morris MR, Astuti D, Maher ER. Perlman syndrome: overgrowth, wilms tumor predisposition and DIS3L2. *Am J Med Genet C Semin Med Genet* (2013) 163C(2):106–13. doi: 10.1002/ajmg.c.31358

36. Neri G, Martini-Neri ME, Katz BE, Opitz JM. The perlman syndrome: Familial renal dysplasia with wilms tumor, fetal gigantism and multiple congenital anomalies. *Am J Med Genet* (1984) 19(1):195–207. doi: 10.1002/ajmg.1320190120

37. Foster A, Chalot B, Antoniadi T, Schaefer E, Keelagher R, Ryan G, et al. Kosaki overgrowth syndrome: A novel pathogenic variant in PDGFRB and expansion of the phenotype including cerebrovascular complications. *Clin Genet* (2020) 98(1):19–31. doi: 10.1111/cge.13752

38. Tenorio J, Mansilla A, Valencia M, Martínez-Glez V, Romanelli V, Arias P, et al. A new overgrowth syndrome is due to mutations in RNF125. *Hum Mutat* (2014) 35 (12):1436–41. doi: 10.1002/humu.22689

39. Hussain K, Challis B, Rocha N, Payne F, Minic M, Thompson A, et al. An activating mutation of AKT2 and human hypoglycemia. *Science* (2011) 334(6055):474. doi: 10.1126/science.1210878

40. Arya VB, Flanagan SE, Schober E, Rami-Merhar B, Ellard S, Hussain K. Activating AKT2 mutation: Hypoinsulinemic hypoketotic hypoglycemia. *J Clin Endocrinol Metab* (2014) 99(2):391–4. doi: 10.1210/jc.2013-3228

41. Garg N, Bademci G, Foster J2nd, Sıklar Z, Berberoglu M, Tekin M. MORFAN syndrome: An infantile hypoinsulinemic hypoketotic hypoglycemia due to an AKT2 mutation. *J Pediatr* (2015) 167(2):489–91. doi: 10.1016/j.jpeds.2015.04.069

42. Ochoa Molina MF, Poggi H, De Toro V, Mendoza C, Hussain K. Facial dysmorphic features in a patient with nonketotic hypoglycemia and a pathogenic variant in the AKT2 gene. *AACE Clin Case Rep* (2021) 8(3):109–12. doi: 10.1016/j.aace.2021.11.006

43. Leiter SM, Parker VER, Welters A, Knox R, Rocha N, Clark G, et al. Hypoinsulinaemic, hypoketotic hypoglycaemia due to mosaic genetic activation of PI3-kinase. *Eur J Endocrinol* (2017) 177(2):175–86. doi: 10.1530/EJE-17-0132

44. McDermott JH, Hickson N, Banerjee I, Murray PG, Ram D, Metcalfe K, et al. Hypoglycaemia represents a clinically significant manifestation of PIK3CA- and CCND2-associated segmental overgrowth. *Clin Genet* (2018) 93(3):687–92. doi: 10.1111/cge.13145

45. Saito T, Nakane T, Narusawa M, Yagasaki H, Nemoto A, Naito A, et al. Giant umbilical cord and hypoglycemia in an infant with Proteus syndrome. *Am J Med Genet A* (2018) 176(5):1222–4. doi: 10.1002/ajmg.a.38674

46. Liu J, Ding G, Zou K, Jiang Z, Zhang J, Lu Y, et al. Sun Z.cGenome sequencing analysis of a family with a child displaying severe abdominal distention and recurrent hypoglycemia. *Mol Genet Genom Med* (2020) 8(3):e1130. doi: 10.1002/mgg3.1130

47. Kinross KM, Montgomery KG, Mangiafico SP, Hare LM, Kleinschmidt M, Bywater MJ, et al. Ubiquitous expression of the Pik3caH1047R mutation promotes hypoglycemia, hypoinsulinemia, and organomegaly. *FASEB J* (2015) 29(4):1426–34. doi: 10.1096/fj.14-262782

48. Dushar M, Nowaczyk J, Pyrżak B, Akopyan H, Śmigiel R, Walczak A, et al. Efficacy and safety of sirolimus therapy in familial hypoinsulinemic hypoglycemia caused by AKT2 mutation inherited from the mosaic father. *Eur J Med Genet* (2021) 64 (12):104368. doi: 10.1016/j.ejmg.2021.104368

49. Adam MP, Hudgins L, Hannibal M. Kabuki syndrome. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2011).

50. Yap KL, Johnson AEK, Fischer D, Kandikatla P, Deml J, Nelakuditi V, et al. Congenital hyperinsulinism as the presenting feature of kabuki syndrome: Clinical and molecular characterization of 9 affected individuals. *Genet Med* (2019) 21(1):233–42. doi: 10.1038/s41436-018-0013-9

51. De Leon DD, Stanley CA. Congenital hypoglycemia disorders: New aspects of etiology, diagnosis, treatment and outcomes: Highlights of the proceedings of the congenital hypoglycemia disorders symposium, Philadelphia April 2016. *Pediatr Diabetes* (2017) 18(1):3–9. doi: 10.1111/pedi.12453

52. Geneviève D, Amiel J, Viot G, Le Merrer M, Sanlaville D, Urtizberea A, et al. Atypical findings in kabuki syndrome: report of 8 patients in a series of 20 and review of the literature. *Am J Med Genet A* (2004) 129A(1):64–8. doi: 10.1002/ajmg.a.30144

53. Subbarayan A, Hussain K. Hypoglycemia in kabuki syndrome. *Am J Med Genet A* (2014) 164A(2):467–71. doi: 10.1002/ajmg.a.36256

54. Gohda Y, Oka S, Matsunaga T, Watanabe S, Yoshiura K, Kondoh T, et al. Neonatal case of novel KMT2D mutation in kabuki syndrome with severe hypoglycemia. *Pediatr Int* (2015) 57(4):726–8. doi: 10.1111/ped.12574

55. Gole H, Chuk R, Coman D. Persistent hyperinsulinism in kabuki syndrome 2: Case report and literature review. *Clin Pract* (2016) 6(3):848. doi: 10.4081/cp.2016.848

56. Phetthong T, Tim-Aroon T, Khongkrapan A, Poomthavorn P, Wattanasirichaigoon D. Kabuki syndrome with midgut malrotation and hyperinsulinemic hypoglycemia: A rare co-occurrence from Thailand. *Am J Med Genet A* (2020) 182(8):1873–6. doi: 10.1002/ajmg.a.61723

57. Mısırlıgil M, Yıldız Y, Akın O, Odabaşı Güneş S, Arslan M, Ünay B. A rare cause of hyperinsulinemic hypoglycemia: Kabuki syndrome. *J Clin Res Pediatr Endocrinol* (2021) 13(4):452–5. doi: 10.4274/jcrpe.galenos.2020.2020.0065

58. Piro E, Schierz IAM, Antona V, Pappalardo MP, Giuffrè M, Serra G, et al. Neonatal hyperinsulinemic hypoglycemia: Case report of kabuki syndrome due to a novel KMT2D splicing-site mutation. *Ital J Pediatr* (2020) 46(1):136. doi: 10.1186/s13052-020-00902-8

59. Salguero MV, Chan K, Greeley SAW, Dyamenahalli U, Waggoner D, Del Gaudio D, et al. Novel KDM6A kabuki syndrome mutation with hyperinsulinemic hypoglycemia and pulmonary hypertension requiring ECMO. *J Endocr Soc* (2022) 6(4): bvac015. doi: 10.1210/jendso/bvac015

60. Hoermann H, El-Rifai O, Schebek M, Lodefalk M, Brusgaard K, Bachmann N, et al. Comparative meta-analysis of kabuki syndrome with and without hyperinsulinaemic hypoglycaemia. *Clin Endocrinol (Oxf)* (2020) 93(3):346–54. doi: 10.1111/cen.14267

61. Faundes V, Goh S, Akilapa R, Bezuidenhout H, Bjornsson HT, Bradley L, et al. Clinical delineation, sex differences, and genotype-phenotype correlation in pathogenic KDM6A variants causing X-linked kabuki syndrome type 2. *Genet Med* (2021) 23 (7):1202–10. doi: 10.1038/s41436-021-01119-8

62. Gripp KW, Rauen KA. Costello Syndrome. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2006).

63. Myers A, Bernstein JA, Brennan ML, Curry C, Esplin ED, Fisher J, et al. Perinatal features of the RASopathies: Noonan syndrome, cardiofaciocutaneous syndrome and Costello syndrome. *Am J Med Genet A* (2014) 164A(11):2814–21. doi: 10.1002/ajmg.a.36737

64. Alexander S, Ramadan D, Alkhayyat H, Al-Sharkawi I, Backer KC, El-Sabban F, et al. Costello Syndrome and hyperinsulinemic hypoglycemia. *Am J Med Genet A* (2005) 139(3):227–30. doi: 10.1002/ajmg.a.31011

65. Sheffield BS, Yip S, Ruchelli ED, Dunham CP, Sherwin E, Brooks PA, et al. Fatal congenital hypertrophic cardiomyopathy and a pancreatic nodule morphologically identical to focal lesion of congenital hyperinsulinism in an infant with costello syndrome: Case report and review of the literature. *Pediatr Dev Pathol* (2015) 18 (3):237–44. doi: 10.2350/14-07-1525-CR.1

66. Vuralli D, Kosukcu C, Taskiran E, Simsek-Kiper PO, Utine GE, Boduroglu K, et al. Hyperinsulinemic hypoglycemia in a patient with Costello syndrome: An etiology to consider in hypoglycemia. *Mol Syndromol* (2020) 11(4):207–16. doi: 10.1159/000510171

67. Kerr B, Delrue MA, Sigaudy S, Perveen R, Marche M, Burgelin I, et al. Genotype-phenotype correlation in Costello syndrome: HRAS mutation analysis in 43 cases. *J Med Genet* (2006) 43(5):401–5. doi: 10.1136/jmg.2005.040352

68. Gripp KW, Robbins KM, Sheffield BS, Lee AF, Patel MS, Yip S, et al. Paternal uniparental disomy 11p15.5 in the pancreatic nodule of an infant with Costello syndrome: Shared mechanism for hyperinsulinemic hypoglycemia in neonates with Costello and beckwith-wiedemann syndrome and somatic loss of heterozygosity in Costello syndrome driving clonal expansion. *Am J Med Genet A* (2016) 170(3):559–64. doi: 10.1002/ajmg.a.37471

69. Kratz CP, Steinemann D, Niemeyer CM, Schlegelberger B, Koscielniak E, Kontny U, et al. Uniparental disomy at chromosome 11p15.5 followed by HRAS mutations in embryonal rhabdomyosarcoma: lessons from Costello syndrome. *Hum Mol Genet* (2007) 16(4):374–9. doi: 10.1093/hmg/ddl458

70. Leoni C, Massese M, Gervasoni J, Primiano A, Giorgio V, Onesimo R, et al. Metabolic profiling of Costello syndrome: Insights from a single-center cohort. *Eur J Med Genet* (2022) 65(3):104439. doi: 10.1016/j.ejmg.2022.104439

71. Oba D, Inoue SI, Miyagawa-Tomita S, Nakashima Y, Niihori T, Yamaguchi S, et al. Mice with an oncogenic HRAS mutation are resistant to high-fat diet-induced obesity and exhibit impaired hepatic energy homeostasis. *EBioMedicine* (2018) 27:138–50. doi: 10.1016/j.ebiom.2017.11.029

72. Alkhayyat H, Christesen HB, Steer J, Stewart H, Brusgaard K, Hussain K. Mosaic turner syndrome and hyperinsulinaemic hypoglycaemia. *J Pediatr Endocrinol Metab* (2006) 19(12):1451–7. doi: 10.1515/jpem.2006.19.12.1451

73. Cappella M, Graziani V, Pragliola A, Sensi A, Hussain K, Muratori C, et al. Hyperinsulinemic hypoglycaemia in a turner syndrome with ring (X). *Case Rep Pediatr* (2015) 2015:561974. doi: 10.1155/2015/561974

74. Pietzner V, Weigel JF, Wand D, Merkenschlager A, Bernhard MK. Low-level hyperinsulinism with hypoglycemic spells in an infant with mosaic turner syndrome and mild kabuki-like phenotype: A case report and review of the literature. *J Pediatr Endocrinol Metab* (2014) 27(1-2):165–70. doi: 10.1515/jpem-2013-0090

75. Gibson CE, Boodhansingh KE, Li C, Conlin L, Chen P, Becker SA, et al. Congenital hyperinsulinism in infants with turner syndrome: Possible association with monosomy X and KDM6A haploinsufficiency. *Horm Res Paediatr* (2018) 89(6):413–22. doi: 10.1159/000488347

76. Salgin B, Amin R, Yuen K, Williams RM, Murgatroyd P, Dunger DB. Insulin resistance is an intrinsic defect independent of fat mass in women with turner's syndrome. *Horm Res* (2006) 65(2):69–75. doi: 10.1159/000090907

77. Sheanon N, Elder D, Khoury J, Casnellie L, Gutmark-Little I, Cernich J, et al. Increased prevalence of beta-cell dysfunction despite normal HbA1c in youth and young adults with turner syndrome. *Horm Res Paediatr* (2021) 94(7-8):297–306. doi: 10.1159/000520233

78. Banerjee I, Senniappan S, Laver TW, Caswell R, Zenker M, Mohnike K, et al. Refinement of the critical genomic region for congenital hyperinsulinism in the chromosome 9p deletion syndrome. *Wellcome Open Res* (2020) 4:149. doi: 10.12688/wellcomeopenres.15465.2

79. Bitner-Glindzicz M, Lindley KJ, Rutland P, Blaydon D, Smith VV, Milla PJ, et al. A recessive contiguous gene deletion causing infantile hyperinsulinism, enteropathy and deafness identifies the usher type 1C gene. *Nat Genet* (2000) 26(1):56–60. doi: 10.1038/79178

80. Hussain K, Bitner-Glindzicz M, Blaydon D, Lindley KJ, Thompson DA, Kriss T, et al. Infantile hyperinsulinism associated with enteropathy, deafness and renal tubulopathy: clinical manifestations of a syndrome caused by a contiguous gene deletion located on chromosome 11p. *J Pediatr Endocrinol Metab* (2004) 17 (12):1613–21. doi: 10.1515/jpem.2004.17.12.1613

81. Al Mutair AN, Brusgaard K, Bin-Abbas B, Hussain K, Felimban N, Al Shaikh A, et al. Heterogeneity in phenotype of usher-congenital hyperinsulinism syndrome: hearing loss, retinitis pigmentosa, and hyperinsulinemic hypoglycemia ranging from severe to mild with conversion to diabetes. *Diabetes Care* (2013) 36(3):557–61. doi: 10.2337/dc12-1174

82. Smith VS, Giacoia GP. Hyperinsulinaemic hypoglycaemia in an infant with mosaic trisomy 13. *J Med Genet* (1985) 22(3):228–30. doi: 10.1136/jmg.22.3.228

83. Bellaton E, Aizenfisz S, Saizou C, Baumann C, Beaufils F, Dauger S. Trisomy 13 and neonatal hyperinsulinism. *Arch Pediatr* (2002) 9(11):1210–1. doi: 10.1016/s0929-693x(02)00099-4

84. Tamame T, Hori N, Homma H, Yoshida R, Inokuchi M, Kosaki K, et al. Hyperinsulinemic hypoglycemia in a newborn infant with trisomy 13. *Am J Med Genet A* (2004) 129A(3):321–2. doi: 10.1002/ajmg.a.30147

85. Shiu YK, Lo FM, Lam TS, Chow CB. A case of partial trisomy 13 presenting with hyperinsulinaemic hypoglycaemia. *Hong Kong Med J* (2003) 9(5):381–3.

86. Kostopoulou E, Dastamani A, Caiulo S, Antell H, Flanagan SE, Shah P. Hyperinsulinaemic hypoglycaemia: A new presentation of 16p11.2 deletion syndrome. *Clin Endocrinol (Oxf)* (2019) 90(5):766–9. doi: 10.1111/cen.13951

87. Hoytema van Konijnenburg EMM, Luirink IK, Schagen SEE, Engelen M, Berendse K, Poll-The BT, et al. Hyperinsulinism in a patient with a zellweger spectrum disorder and a 16p11.2 deletion syndrome. *Mol Genet Metab Rep* (2020) 23:100590. doi: 10.1016/j.ymgmr.2020.100590

88. Taylor CM, Smith R, Lehman C, Mitchel MW, Singer K, Weaver WC, et al. 16p11.2 recurrent deletion. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2009).

89. Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, et al. Large, Rare chromosomal deletions associated with severe early-onset obesity. *Nature* (2010) 463(7281):666–70. doi: 10.1038/nature08689

90. Szelest M, Stefaniak M, Ręka G, Jaszczuk I, Lejman M. Three case reports of patients indicating the diversity of molecular and clinical features of 16p11.2 microdeletion anomaly. *BMC Med Genomics* (2021) 14(1):76. doi: 10.1186/s12920-021-00929-8

91. Baple E, Palmer R, Hennekam RC. A microdeletion at 12q24.31 can mimic beckwith-wiedemann syndrome neonatally. *Mol Syndromol* (2010) 1(1):42–5. doi: 10.1159/000275671

92. Hewat TI, Laver TW, Houghton JAL, Männistö JME, Alvi S, Brearey SP, et al. Increased referrals for congenital hyperinsulinism genetic testing in children with trisomy 21 reflects the high burden of non-genetic risk factors in this group. *Pediatr Diabetes* (2022) 23(4):457–61. doi: 10.1111/pedi.13333

93. Männistö JME, Jääskeläinen J, Otonkoski T, Huopio H. Long-term outcome and treatment in persistent and transient congenital hyperinsulinism: A Finnish population-based study. *J Clin Endocrinol Metab* (2021) 106(4):e1542–51. doi: 10.1210/clinem/dgab024

94. Giri D, Patil P, Hart R, Didi M, Senniappan S. Congenital hyperinsulinism and Poland syndrome in association with 10p13-14 duplication. *Endocrinol Diabetes Metab Case Rep* (2017) 2017:16–0125. doi: 10.1530/EDM-16-0125

95. Sparks SE, Krasnewich DM. Congenital disorders of n-linked glycosylation and multiple pathway overview. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2005).

96. Pajusalu S, Vals MA, Mihkla L, Šamarina U, Kahre T, Õunap K. The estimated prevalence of n-linked congenital disorders of glycosylation across various populations based on allele frequencies in general population databases. *Front Genet* (2021) 12:719437. doi: 10.3389/fgene.2021.719437

97. Vajravelu ME, De León DD. Genetic characteristics of patients with congenital hyperinsulinism. *Curr Opin Pediatr* (2018) 30(4):568–75. doi: 10.1097/MOP.0000000000000645

98. Vuralli D, Yildiz Y, Ozon A, Dursun A, Gonc N, Tokatlı A, et al. Alikasifoglu A.Hyperinsulinism may be underreported in hypoglycemic patients with phosphomannomutase 2 deficiency. *J Clin Res Pediatr Endocrinol* (2022) 14(3):275–286. doi: 10.4274/jcrpe.galenos.2021.2021-10-14

99. Čechová A, Altassan R, Borgel D, Bruneel A, Correia J, Girard M, et al. Consensus guideline for the diagnosis and management of mannose phosphate isomerase-congenital disorder of glycosylation. *J Inherit Metab Dis* (2020) 43(4):671–93. doi: 10.1002/jimd.12241

100. de Lonlay P, Cuer M, Vuillaumier-Barrot S, Beaune G, Castelnau P, Kretz M, et al. Hyperinsulinemic hypoglycemia as a presenting sign in phosphomannose isomerase deficiency: A new manifestation of carbohydrate-deficient glycoprotein syndrome treatable with mannose. *J Pediatr* (1999) 135(3):379–83. doi: 10.1016/s0022-3476(99)70139-3

101. Deeb A, Al Amoodi A. A novel homozygous mutation in the mannose phosphate isomerase gene causing congenital disorder of glycation and hyperinsulinemic hypoglycemia in an infant. *Clin Case Rep* (2018) 6(3):479–83. doi: 10.1002/ccr3.1387

102. Wong SY, Beamer LJ, Gadomski T, Honzik T, Mohamed M, Wortmann SB, et al. Defining the phenotype and assessing severity in phosphoglucomutase-1 deficiency. *J Pediatr* (2016) 175:130–136.e8. doi: 10.1016/j.jpeds.2016.04.021

103. Sun L, Eklund EA, Chung WK, Wang C, Cohen J, Freeze HH. Congenital disorder of glycosylation id presenting with hyperinsulinemic hypoglycemia and islet cell hyperplasia. *J Clin Endocrinol Metab* (2005) 90(7):4371–5. doi: 10.1210/jc.2005-0250

104. Conti LR, Radeke CM, Vandenberg CA. Membrane targeting of ATP-sensitive potassium channel. effects of glycosylation on surface expression. *J Biol Chem* (2002) 277(28):25416–22. doi: 10.1074/jbc.M203109200

105. Cabezas OR, Flanagan SE, Stanescu H, García-Martínez E, Caswell R, Lango-Allen H, et al. Polycystic kidney disease with hyperinsulinemic hypoglycemia caused by a promoter mutation in phosphomannomutase 2. *J Am Soc Nephrol* (2017) 28(8):2529–39. doi: 10.1681/ASN.2016121312

106. Soares AR, Figueiredo CM, Quelhas D, Silva ES, Freitas J, Oliveira MJ, et al. Hyperinsulinaemic hypoglycaemia and polycystic kidney disease - a rare case concerning PMM2 gene pleiotropy. *Eur Endocrinol* (2020) 16(1):66–8. doi: 10.17925/EE.2020.16.1.66

107. Chen C, Sang Y. Phosphomannomutase 2 hyperinsulinemia: Recent advances of genetic pathogenesis, diagnosis, and management. *Front Endocrinol (Lausanne)* (2023) 13:1102307. doi: 10.3389/fendo.2022.1102307

108. Napolitano C, Timothy KW, Bloise R, Priori SG. CACNA1C-related disorders. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2006).

109. Splawski I, Timothy KW, Sharpe LM, Decher N, Kumar P, Bloise R, et al. Ca (V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* (2004) 119(1):19–31. doi: 10.1016/j.cell.2004.09.011

110. Dufendach KA, Timothy K, Ackerman MJ, Blevins B, Pflaumer A, Etheridge S, et al. Clinical outcomes and modes of death in Timothy syndrome: A multicenter

international study of a rare disorder. *JACC Clin Electrophysiol* (2018) 4(4):459–66. doi: 10.1016/j.jacep.2017.08.007

111. Kummer S, Rinné S, Seemann G, Bachmann N, Timothy K, Thornton PS, et al. Hyperinsulinemic hypoglycemia associated with a CaV1.2 variant with mixed gain- and loss-of-Function effects. *Int J Mol Sci* (2022) 23(15):8097. doi: 10.3390/ijms23158097

112. Flanagan SE, Vairo F, Johnson MB, Caswell R, Laver TW, Lango Allen H, et al. A CACNA1D mutation in a patient with persistent hyperinsulinaemic hypoglycaemia, heart defects, and severe hypotonia. *Pediatr Diabetes* (2017) 18(4):320–3. doi: 10.1111/pedi.12512

113. De Mingo Alemany MC, Mifsud Grau L, Moreno Macián F, Ferrer Lorente B, León Cariñena SA. *de novo* CACNA1D missense mutation in a patient with congenital hyperinsulinism, primary hyperaldosteronism and hypotonia. *Channels (Austin)* (2020) 14(1):175–80. doi: 10.1080/19336950.2020.1761171

114. Iwashima Y, Pugh W, Depaoli AM, Takeda J, Seino S, Bell GI, et al. Expression of calcium channel mRNAs in rat pancreatic islets and downregulation after glucose infusion. *Diabetes* (1993) 42(7):948–55. doi: 10.2337/diab.42.7.948

115. Liu G, Jacobo SM, Hilliard N, Hockerman GH. Differential modulation of Cav1.2 and Cav1.3-mediated glucose-stimulated insulin secretion by cAMP in INS-1 cells: Distinct roles for exchange protein directly activated by cAMP 2 (Epac2) and protein kinase a. *J Pharmacol Exp Ther* (2006) 318(1):152–60. doi: 10.1124/jpet.105.097477

116. Nitert MD, Nagorny CL, Wendt A, Eliasson L, Mulder H. CaV1.2 rather than CaV1.3 is coupled to glucose-stimulated insulin secretion in INS-1 832/13 cells. *J Mol Endocrinol* (2008) 41(1):1–11. doi: 10.1677/JME-07-0133

117. Torekov SS, Iepsen E, Christiansen M, Linneberg A, Pedersen O, Holst JJ, et al. KCNQ1 long QT syndrome patients have hyperinsulinemia and symptomatic hypoglycemia. *Diabetes* (2014) 63(4):1315–25. doi: 10.2337/db13-1454

118. Lubberding AF, Zhang J, Lundh M, Nielsen TS, Søndergaard MS, Villadsen M, et al. Age-dependent transition from islet insulin hypersecretion to hyposecretion in mice with the long QT-syndrome loss-of-function mutation Kcnq1-A340V. *Sci Rep* (2021) 11(1):12253. doi: 10.1038/s41598-021-90452-8

119. Hyltén-Cavallius L, Iepsen EW, Wewer Albrechtsen NJ, Svendstrup M, Lubberding AF, Hartmann B, et al. Patients with long-QT syndrome caused by impaired hERG-encoded Kv11.1 potassium channel have exaggerated endocrine pancreatic and incretin function associated with reactive hypoglycemia. *Circulation* (2017) 135(18):1705–19. doi: 10.1161/CIRCULATIONAHA.116.024279

120. Giri D, Vignola ML, Gualtieri A, Scagliotti V, McNamara P, Peak M, et al. Novel FOXA2 mutation causes hyperinsulinism, hypopituitarism with craniofacial and endoderm-derived organ abnormalities. *Hum Mol Genet* (2017) 26(22):4315–26. doi: 10.1093/hmg/ddx318

121. Vajravelu ME, Chai J, Krock B, Baker S, Langdon D, Alter C, et al. Congenital hyperinsulinism and hypopituitarism attributable to a mutation in FOXA2. *J Clin Endocrinol Metab* (2018) 103(3):1042–7. doi: 10.1210/jc.2017-02157

122. Kaygusuz SB, Arslan Ates E, Vignola ML, Volkan B, Geckinli BB, Turan S, et al. Dysgenesis and dysfunction of the pancreas and pituitary due to FOXA2 gene defects. *J Clin Endocrinol Metab* (2021) 106(10):e4142–54. doi: 10.1210/clinem/dgab352

123. Gao N, LeLay J, Vatamaniuk MZ, Rieck S, Friedman JR, Kaestner KH. Dynamic regulation of Pdx1 enhancers by Foxa1 and Foxa2 is essential for pancreas development. *Genes Dev* (2008) 22(24):3435–48. doi: 10.1101/gad.1752608

124. Lantz KA, Vatamaniuk MZ, Brestelli JE, Friedman JR, Matschinsky FM, Kaestner KH. Foxa2 regulates multiple pathways of insulin secretion. *J Clin Invest* (2004) 114(4):512–20. doi: 10.1172/JCI21149

125. Silva JP, von Meyenn F, Howell J, Thorens B, Wolfrum C, Stoffel M. Regulation of adaptive behaviour during fasting by hypothalamic Foxa2. *Nature* (2009) 462 (7273):646–50. doi: 10.1038/nature08589

126. Sund NJ, Vatamaniuk MZ, Casey M, Ang SL, Magnuson MA, Stoffers DA, et al. Tissue-specific deletion of Foxa2 in pancreatic beta cells results in hyperinsulinemic hypoglycemia. *Genes Dev* (2001) 15(13):1706–15. doi: 10.1101/gad.901601

127. Gregory LC, Ferreira CB, Young-Baird SK, Williams HJ, Harakalova M, van Haaften G, et al. Impaired EIF2S3 function associated with a novel phenotype of X-linked hypopituitarism with glucose dysregulation. *EBioMedicine* (2019) 42:470–80. doi: 10.1016/j.ebiom.2019.03.013

128. Stanik J, Skopkova M, Stanikova D, Brennerova K, Barak L, Ticha L, et al. Neonatal hypoglycemia, early-onset diabetes and hypopituitarism due to the mutation in EIF2S3 gene causing MEHMO syndrome. *Physiol Res* (2018) 67(2):331–7. doi: 10.33549/physiolres.933689

129. Ygberg S, Lindstrand A. Expanding the phenotype of the recurrent truncating eIF2γ pathogenic variant p. (Ile465Serfs*4) identified in two brothers with MEHMO syndrome. *Clin Case Rep* (2022) 10(6):e5989. doi: 10.1002/ccr3.5989

130. Hopkins E, Stark J, Mosquera RA. Central congenital hypoventilation syndrome associated with hypoglycemia and seizure. *Respir Med Case Rep* (2016) 20:75–6. doi: 10.1016/j.rmcr.2016.12.004

131. Hennewig U, Hadzik B, Vogel M, Meissner T, Goecke T, Peters H, et al. Congenital central hypoventilation syndrome with hyperinsulinism in a preterm infant. *J Hum Genet* (2008) 53(6):573–7. doi: 10.1007/s10038-008-0275-1

132. Farina MI, Scarani R, Po' C, Agosto C, Ottonello G, Benini F. Congenital central hypoventilation syndrome and hypoglycaemia. *Acta Paediatr* (2012) 101(2): e92–6. doi: 10.1111/j.1651-2227.2011.02533.x

133. Marics G, Amiel J, Vatai B, Lódi C, Mikos B, Tóth-Heyn P. Autonomic dysfunction of glucose homoeostasis in congenital central hypoventilation syndrome. *Acta Paediatr* (2013) 102(4):e178–80. doi: 10.1111/apa.12125

134. Musthaffa YM, Goyal V, Harris MA, Kapur N, Leger J, Harris M. Dysregulated glucose homeostasis in congenital central hypoventilation syndrome. *J Pediatr Endocrinol Metab* (2018) 31(12):1325–33. doi: 10.1515/jpem-2018-0086

135. Wyatt D. Transient hypoglycemia with hyperinsulinemia in a newborn infant with Rubinstein-taybi syndrome. *Am J Med Genet* (1990) 37(1):103–5. doi: 10.1002/ajmg.1320370124

136. Costain G, Kannu P, Bowdin S. Genome-wide sequencing expands the phenotypic spectrum of EP300 variants. *Eur J Med Genet* (2018) 61(3):125–9. doi: 10.1016/j.ejmg.2017.11.002

137. Welters A, El-Khairi R, Dastamani A, Bachmann N, Bergmann C, Gilbert C, et al. Persistent hyperinsulinaemic hypoglycaemia in children with Rubinstein-taybi syndrome. *Eur J Endocrinol* (2019) 181(2):121–8. doi: 10.1530/EJE-19-0119

138. Wild KT, Nomakuchi TT, Sheppard SE, Leavens KF, De León DD, Zackai EH. Hyperinsulinism in an individual with an EP300 variant of Rubinstein-taybi syndrome. *Am J Med Genet A* (2021) 185(4):1251–5. doi: 10.1002/ajmg.a.62085

139. Cohen JL, Schrier Vergano SA, Mazzola S, Strong A, Keena B, McDougall C, et al. EP300-related Rubinstein-taybi syndrome: Highlighted rare phenotypic findings and a genotype-phenotype meta-analysis of 74 patients. *Am J Med Genet A* (2020) 182 (12):2926–38. doi: 10.1002/ajmg.a.61883

140. Wong CK, Wade-Vallance AK, Luciani DS, Brindle PK, Lynn FC, Gibson WT. The p300 and CBP transcriptional coactivators are required for β-cell and α-cell proliferation. *Diabetes* (2018) 67(3):412–22. doi: 10.2337/db17-0237

141. Sekiguchi K, Itonaga T, Maeda T, Fukami M, Yorifuji T, Ihara K. A case of CHARGE syndrome associated with hyperinsulinemic hypoglycemia in infancy. *Eur J Med Genet* (2018) 61(6):312–4. doi: 10.1016/j.ejmg.2018.01.008

142. van Ravenswaaij-Arts CM, Hefner M, Blake K, Martin DM. CHD7 disorder. In: Adam MP, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. *GeneReviews*. Seattle (WA: University of Washington, Seattle (2006).

143. Imaizumi K, Nakamura M, Masuno M, Makita Y, Kuroki Y. Hypoglycemia in coffin-siris syndrome. *Am J Med Genet* (1995) 59(1):49–50. doi: 10.1002/ajmg.1320590111

144. Vals MA, Õiglane-Shlik E, Nõukas M, Shor R, Peet A, Kals M, et al. Coffin-siris syndrome with obesity, macrocephaly, hepatomegaly and hyperinsulinism caused by a mutation in the ARID1B gene. *Eur J Hum Genet* (2014) 22(11):1327–9. doi: 10.1038/ejhg.2014.25

145. Sonmez FM, Uctepe E, Gunduz M, Gormez Z, Erpolat S, Oznur M, et al. Coffin-siris syndrome with café-au-lait spots, obesity and hyperinsulinism caused by a mutation in the ARID1B gene. *Intractable Rare Dis Res* (2016) 5(3):222–6. doi: 10.5582/irdr.2014.01040

146. Flanagan SE, Kapoor RR, Mali G, Cody D, Murphy N, Schwahn B, et al. Diazoxide-responsive hyperinsulinemic hypoglycemia caused by HNF4A gene mutations. *Eur J Endocrinol* (2010) 162(5):987–92. doi: 10.1530/EJE-09-0861

147. Stanescu DE, Hughes N, Kaplan B, Stanley CA, De León DD. Novel presentations of congenital hyperinsulinism due to mutations in the MODY genes: HNF1A and HNF4A. *J Clin Endocrinol Metab* (2012) 97(10):E2026–30. doi: 10.1210/jc.2012-1356

148. Hamilton AJ, Bingham C, McDonald TJ, Cook PR, Caswell RC, Weedon MN, et al. The HNF4A R76W mutation causes atypical dominant fanconi syndrome in addition to a β cell phenotype. *J Med Genet* (2014) 51(3):165–9. doi: 10.1136/jmedgenet-2013-102066

149. Liu J, Shen Q, Li G, Xu H. HNF4A-related fanconi syndrome in a Chinese patient: a case report and review of the literature. *J Med Case Rep* (2018) 12(1):203. doi: 10.1186/s13256-018-1740-x

150. Soden SE, Saunders CJ, Willig LK, Farrow EG, Smith LD, Petrikin JE, et al. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med* (2014) 6(265):265ra168. doi: 10.1126/scitranslmed.3010076

151. Halloun R, Habib C, Ekhilevitch N, Weiss R, Tiosano D, Cohen M. Expanding the spectrum of endocrinopathies identified in schaaf-yang syndrome - a case report and review of the literature. *Eur J Med Genet* (2021) 64(8):104252. doi: 10.1016/j.ejmg.2021.104252

152. Gillis D, Krishnamohan A, Yaacov B, Shaag A, Jackman JE, Elpeleg O. TRMT10A dysfunction is associated with abnormalities in glucose homeostasis, short stature and microcephaly. *J Med Genet* (2014) 51(9):581–6. doi: 10.1136/jmedgenet-2014-102282

153. Igoillo-Esteve M, Genin A, Lambert N, Désir J, Pirson I, Abdulkarim B, et al. tRNA methyltransferase homolog gene TRMT10A mutation in young onset diabetes and primary microcephaly in humans. *PloS Genet* (2013) 9(10):e1003888. doi: 10.1371/journal.pgen.1003888

154. Zeiad RKHM, Ferren EC, Young DD, De Lancy SJ, Dedousis D, Schillaci LA, et al. A novel homozygous missense mutation in the YARS gene: Expanding the

phenotype of YARS multisystem disease. *J Endocr Soc* (2021) 5(2):bvaa196. doi: 10.1210/jendso/bvaa196

155. Williams KB, Brigatti KW, Puffenberger EG, Gonzaga-Jauregui C, Griffin LB, Martinez ED, et al. Homozygosity for a mutation affecting the catalytic domain of tyrosyl-tRNA synthetase (YARS) causes multisystem disease. *Hum Mol Genet* (2019) 28(4):525–38. doi: 10.1093/hmg/ddy344

156. Baumann U, Preece MA, Green A, Kelly DA, McKiernan PJ. Hyperinsulinism in tyrosinaemia type I. *J Inherit Metab Dis* (2005) 28(2):131–5. doi: 10.1007/s10545-005-5517-1

157. Sethuram S, Sperling MA, Gujral J, Romero CJ. Neonatal hyperinsulinism in transient and classical forms of tyrosinemia. *Orphanet J Rare Dis* (2021) 16(1):190. doi: 10.1186/s13023-020-01642-y

158. Russo P, O'Regan S. Visceral pathology of hereditary tyrosinemia type I. *Am J Hum Genet* (1990) 47(2):317–24.

159. Staufner C, Lindner M, Dionisi-Vici C, Freisinger P, Dobbelaere D, Douillard C, et al. Adenosine kinase deficiency: Expanding the clinical spectrum and evaluating therapeutic options. *J Inherit Metab Dis* (2016) 39(2):273–83. doi: 10.1007/s10545-015-9904-y

160. Kapoor RR, James C, Hussain K. Hyperinsulinism in developmental syndromes. *Endocr Dev* (2009) 14:95–113. doi: 10.1159/000207480

161. Guemes M, Rahman SA, Kapoor RR, Flanagan S, Houghton JAL, Misra S, et al. Hyperinsulinemic hypoglycemia in children and adolescents: Recent advances in understanding of pathophysiology and management. *Rev Endocr Metab Disord* (2020) 21(4):577–97. doi: 10.1007/s11154-020-09548-7

162. Hewat TI, Johnson MB. Flanagan SE. congenital hyperinsulinism: Current laboratory-based approaches to the genetic diagnosis of a heterogeneous disease. *Front Endocrinol (Lausanne)* (2022) 13:873254. doi: 10.3389/fendo.2022.873254

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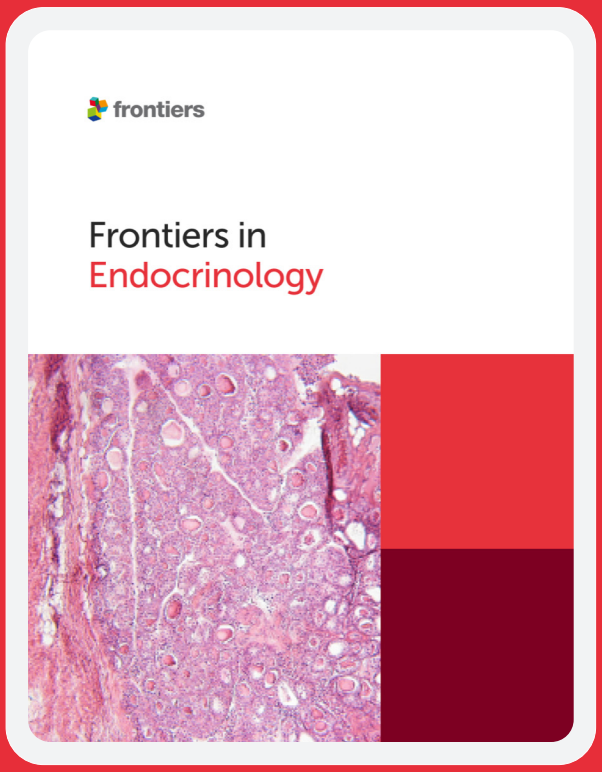

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