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The problem of childhood hypoglycemia, volume II

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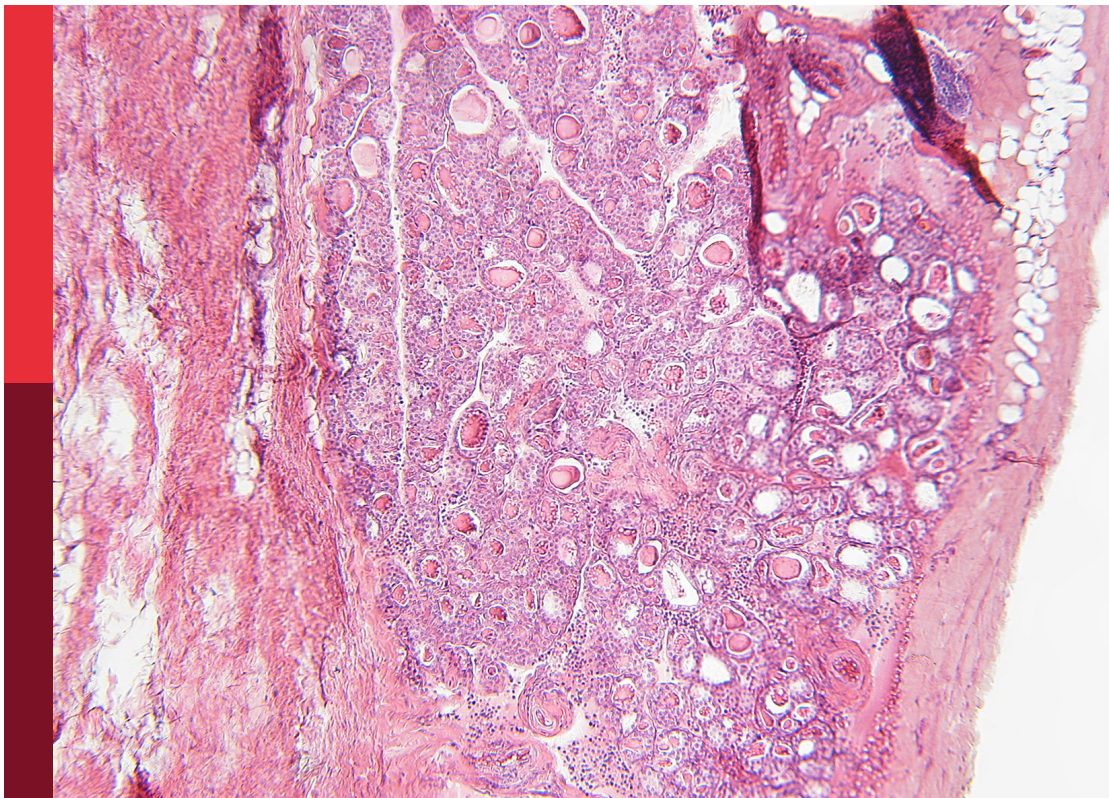

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The problem of childhood hypoglycemia, volume II

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Editorial: The problem of childhood hypoglycemia, volume II

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Editorial on the Research Topic
The problem of childhood hypoglycemia, volume II

Hypoglycemia is common in childhood with long-term neurodisability from irreversible neuronal injury; yet the problem remains obdurately unresolved. The Research Topic series "*The Problem of Childhood Hypoglycemia*" provides an overview of different aspects from translational medicine to patient experience, reflecting a diversity of views from endocrinology, metabolism, genetics, neonatology, radiology and psychology integrated into a multidisciplinary assessment of hypoglycemia.

Despite significant research inquiry, a clear definition of neonatal hypoglycemia has not emerged (Roeper et al.). Mathematical definitions of hypoglycemia, supported by validating studies, have been advocated but they disregard individual variability and the differential effects of the duration and excursion of hypoglycemia. Symptom-based definitions are also ineffective as hypoglycemia is often asymptomatic. The traditional emphasis on glucose and ketones as hypoglycemia metabolites has impeded a search for biomarkers correlating with brain injury, an outcome resonating with a brain magnetic resonance imaging (MRI) study documenting missed neonatal hypoglycemia (Worth et al.). As with data of a non-converging nature, larger studies have been proposed to generate longitudinal neurodevelopmental outcomes to magnify small scale effects (Roeper et al.). However, such studies are cumbersome and predicated on current insufficient understanding, suggesting the need for unbiased hypothesis-free study designs.

While the debate on neonatal hypoglycemia continues unabated, it is important to optimize therapeutic strategies that mitigate against the ill-effects of neuroglycopenic injury, as in the rare disease of congenital hyperinsulinism (CHI) (Figure 1). A standardized practice guideline by specialist centers and utilizing a network model serves as a template to harmonize investigations and treatment plans for CHI patients (Shaikh et al.). This guideline also offers multidisciplinary practical advice on day-to-day parent perspectives such as breast feeding, weaning and follow-up to give a more holistic view of clinical management.

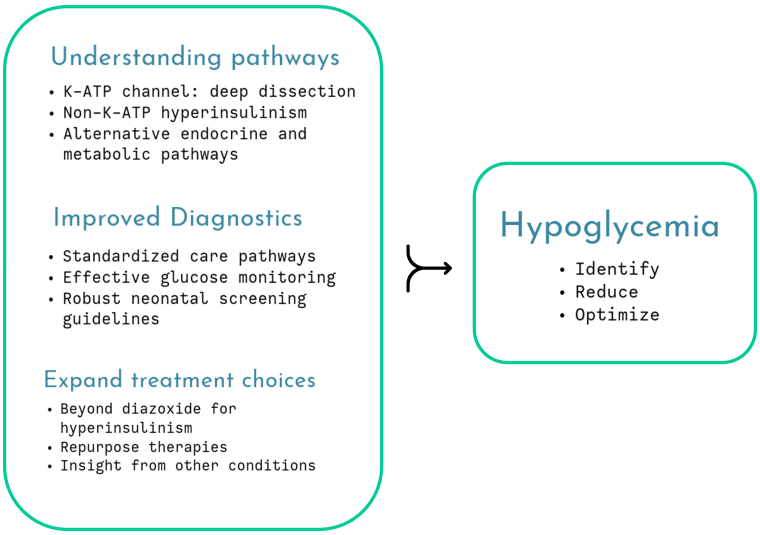

FIGURE 1
Several aspects of childhood hypoglycemia remain unresolved but cumulative research will unravel understanding, enabling improved therapy and monitoring, optimizing glycemic status.

A return of emphasis on the identification on neonatal hypoglycemia (Worth et al.) is presented in a ten-year review of brain MRI. The study identified characteristic radiological features of hypoglycemia in children without a history of neonatal hypoglycemia. Although the study was not designed to establish the true prevalence of missed hypoglycemia, the finding of any missed hypoglycemia escaping from standard neonatal screening procedures is concerning.

An important paradigm that inevitably influences the management of hypoglycemia is the monitoring of glucose levels. In most cases, local investigation algorithms mandate infrequent blood glucose monitoring that fail to account for continuously variable glucose profiles captured by continuous glucose monitoring (CGM) devices. CGM is increasingly recognized in the management of childhood hypoglycemia, albeit with caveats. A comprehensive review of the benefits and challenges in CGM predicts a role for the monitoring of both neonatal hypoglycemia and hyperglycemia (Worth et al.). However, the evidence for wider adoption in childhood hypoglycemia is not strong and calls for rigorous research not only in CHI but also in other disorders such as glycogen storage disorder (GSD) and adrenal insufficiency (Lee et al.).

Considerable attention has been devoted to the understanding of genetic forms of CHI, but less to non-genetic forms of hyperinsulinism, for instance in small for gestational age (SGA) newborn infants. A relatively large observational cohort investigated if diazoxide has greater clinical benefit than a wait/watch approach for hyperinsulinism in SGA (Chandran et al.). Surprisingly, the use of diazoxide did not reduce length of stay in the hospital, although both treatment strategies involved unusually prolonged hospitalization. The authors conclude that watch/wait is as effective as diazoxide, accepting the limitations of variability in a real-world study without longitudinal follow-up to determine neurodevelopmental equivalence.

Another relatively unrecognized cause of non-genetic hyperinsulinism is congenital porto-systemic shunts (CPSS) (van Albada et al.). In a review, illustrated by a case report, the authors present well-argued theoretical constructs describing hepatic bypass as a cause for a surge in insulin and reduced degradation causing hypoglycemia. Treatment for symptomatic CPSS include endovascular or surgical approaches, but slow-release carbohydrates may also prove effective. As with the cautionary narrative of diazoxide in SGA hyperinsulinism (Chandran et al.), diazoxide may increase glucose levels, precipitating post-prandial hypoglycemia in CPSS.

Not surprisingly, the topic dwells on mechanistic insights, both involving (ElSheikh and Shyng) and excluding (Chen and Sang) the pancreatic β-cell K-ATP channel to unravel the pathophysiology of CHI. The K-ATP channel is a central mechanism explaining glucose-insulin coupling ensuring appropriate insulin secretion based on need. Pathogenic variants in the genes coding for the subunits of the channel, *ABCC8*/*KCNJ11*, variably affect channel gating, protein folding, assembly and trafficking, providing a deeper understanding of phenotypic diversity (ElSheikh and Shyng). The authors stress the importance of molecular characterization, gaining insight from novel missense and indel variants as well as non-coding regions to design future therapeutic targets. They suggest laboratory simulation to test dominant effects and pathogenicity and use the latest electron microscopy techniques to describe channel structure in vivid detail but accept that phenotypic variation cannot always be simulated by experiments. Nonetheless, careful dissection of the K-ATP structure and function may eventually identify treatments such as pharmacological chaperones (Figure 1).

In CHI, diazoxide remains first line treatment, although unresponsiveness is common. In a small group of patients with diazoxide-unresponsive CHI, K-ATP pathogenic variants were found in 69%, but no genetic etiology was ascertained in the remainder (Lee et al.). Preponderance of a specific Taiwanese variant, probably due to

a founder effect, was noted and characterized by functional studies demonstrating loss of channel function.

A rare but interesting etiology of CHI has been described in a review on variants in phosphomannomutase 2 (PMM2) (Chen and Sang). *PMM2* variants contribute to abnormal protein glycosylation, including the sulfonylurea receptor (SUR), thereby modulating insulin secretion. Although PMM2 hyperinsulinism has been reported in only 26 patients, mechanistic insight may improve our overall understanding, including the role of *HNF4A*, a known CHI-related gene.

Hypoglycemia in adrenal insufficiency has not received much research attention, even though it is a well-recognized component of life-threatening adrenal crisis in both primary and secondary forms of the condition. A timely review (Lee et al.) summarizes mechanisms of hypoglycemia through loss of counter-regulation, compounded by reduced epinephrine stores and the presence of other hormone deficiencies. The review notes that adrenal hypoglycemia is probably underreported, given the relative high incidence of adrenal crisis in young children. It advocates the need for improved medications and considers glucose monitoring with CGM as a future adjunct to clinical management (Figure 1).

Empagliflozin used in adults with type 2 diabetes has been repurposed for application in the hypoglycemia disorder of GSD (Maiorana et al.). Empagliflozin inhibits renal glucose transporters, preventing glucose reabsorption and reducing glucose levels. In GSD type 1b, accumulation of 1,5 anhydroglucitol interferes with metabolic pathways within neutrophils, causing neutropenia. Here, empagliflozin induced glycosuria facilitates greater excretion of 1,5 anhydroglucitol to preserve neutrophil function. The authors describe clinical benefit in GSD1b, although without direct improvement of hypoglycemia; instead, they seek insight from protective pleiotropic effects on the heart, kidney, liver and neurons in type 2 diabetes, thereby utilizing collateral experiences in the repurposing of medications.

Author contributions

IB: Conceptualization, Writing – original draft, Writing – review & editing. KM: Conceptualization, Writing – review & editing.

Conflict of interest

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Phosphomannomutase 2 hyperinsulinemia: Recent advances of genetic pathogenesis, diagnosis, and management

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Congenital hyperinsulinemia (CHI), is a clinically heterogeneous disorder that presents as a major cause of persistent and recurrent hypoglycemia during infancy and childhood. There are 16 subtypes of CHI-related genes. Phosphomannomutase 2 hyperinsulinemia (PMM2-HI) is an extremely rare subtype which is first reported in 2017, with only 18 families reported so far. This review provides a structured description of the genetic pathogenesis, and current diagnostic and therapeutic advances of PMM2-HI to increase clinicians' awareness of PMM2-HI.

KEYWORDS

congenital disorder of glycosylation, diazoxide, congenital hyperinsulinism, hypoglycemia, phosphomannomutase 2

Search strategy and selection criteria

We searched PubMed for full-text original studies and case reports written in English, to identify reports about the genetic pathogenesis, diagnosis, and management pathophysiology, consequences, and treatment of phosphomannomutase 2 hyperinsulinemia. The search terms used were "hyperinsulinism", "congenital hyperinsulinism", "congenital hypoglycemia", "phosphomannomutase 2", and "PMM2 protein".

Abbreviations: CHI, Congenital hyperinsulinemia; PMM2-HI, Phosphomannomutase 2 hyperinsulinemia; PMM2, Phosphomannomutase 2; KATP-HI, Adenosine triphosphate-sensitive potassium channel hyperinsulinism; GDH-HI, Glutamate dehydrogenase hyperinsulinism; ZNF143, Zinc figure protein 143; HH, hyperinsulinemic hypoglycemia; ARPKD, autosomal recessive polycystic kidney disease; PMM2-CDG, phosphomannomutase-2-congenital disorders of glycosylation; Dol-P-Man, polyphenol-P-oligosaccharide; CDG1a, congenital disorders of glycosylation, type 1a; SUR, sulfonylurea receptor; HGMD, Human Gene Mutation Database; TAD, topologically associated domains; CTCF, CCCTC binding factor; HNF4A, Hepatocyte nuclear factor 4 alpha.

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.

Introduction

Congenital hyperinsulinism (CHI) is one of the principal causes of persistent, recurrent hypoglycemia in infancy and childhood, and is highly genetically and clinically heterogeneous. At least 16 CHI-related pathogenic genes (ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, UCP2, HNF4A, HNF1A, HK1, KCNQ1, CACNA1D, FOXA2, EIF2S3, PGM1, and PMM2) have been identified so far and are involved in the regulation of insulin secretion from pancreatic β-cells (1, 2). Nevertheless, almost 40% of children with CHI still have unidentified genes to date (3). Among the clinical subtypes, adenosine triphosphate-sensitive potassium channel hyperinsulinism (KATP-HI) is the most common and most clinically severe subtype, accounting for approximately 40-50% of CHI patients. Glutamate dehydrogenase hyperinsulinism (GDH-HI) is the second most common type, and the remaining subtypes are extremely rare. Zinc figure protein 143 (ZNF143) has an altered affinity for phosphomannomutase 2 (PMM2) gene promoter due to a variant in the promoter of the PMM2 gene, resulting in abnormal expression of the PMM2, which is a principal N-glycosylation enzyme encoded by the PMM2 gene in the pancreas. Consequently, this results in abnormal insulin secretion from pancreatic β-cells and phosphomannomutase 2 hyperinsulinism (PMM2-HI).

PMM2-HI is one of the extremely rare types of CHI, with approximately at least 18 families and over 26 patients with PMM2-HI reported to date. This review will illustrate the recent advances in genetic pathogenesis, diagnosis, and management regarding PMM2-HI, in an attempt to contribute to clinicians' awareness of the disease, which in turn will facilitate the early diagnosis and management of hypoglycemia in infancy and childhood.

PMM2-HI

In 2004, Müller et al. reported a 5-year-old patient with polycystic kidney disease accompanied by hyperinsulinemic hypoglycemia, and the first clinical association between polycystic kidney disease with hyperinsulinemia was described, but the genetic pathogenesis was not further elucidated (4). In 2007, Cabezas et al. (5) described the association of PMM2 gene promoter variants with hyperinsulinemic hypoglycemia (HH) and autosomal recessive polycystic kidney disease (ARPKD), first proposing the concept of PMM2-HI. Using Sanger sequencing of 17 children with concurrent HH and ARPKD from 11 unrelated European pedigree families, Cabezas et al. identified promoter (c.-167G>T) pure or trans-coding mutations in the PMM2 gene of all patients, with the four patients from the consanguineous families having a pure heterozygous status and the others with a compound heterozygous status. These patients did not present with the characteristic clinical features and laboratory findings of phosphomannomutase-2-congenital disorders of glycosylation (PMM2-CDG, OMIM: #212065), which are the most common disease with variants in the PMM2 but presenting only with symptoms of ARPKD, hepatocytes, and hypoglycemia. Most of these patients present at birth as giant fetuses. The median onset of hypoglycemia in the children was 10 months of age, with only one patient with onset at 4 years of age and the rest within 1 year of age, typically presenting with seizures. Most patients are responding effectively to diazoxide treatment, with a small number of patients spontaneously resolving without treatment (5). Since then, several researchers have identified and supplemented the nucleotide sequences of PMM2 gene promoter mutations in patients diagnosed with HH and ARPKD (6–10). In 2020, Moreno et al. illustrated six patients in four unrelated Spanish families with variants in the PMM2 gene and present with PMM2-HI and ARPKD, all of whom had the heterozygous variant c-167G>T in the PMM2 promoter region. Moreno et al. presented that patients with PMM2 promoter mutations might also carry PMM2-CDG-associated mutations, but only 2 patients suffered from the PMM2-CDG phenotype (7).

PMM2-CDG is a disease that was first proposed in 1997 (11) and can present hyperinsulinemia, but most of the currently known literature on PMM2-CDG only mentions the hypoglycemic phenotype and insulin level at the time of hypoglycemia was not demonstrated detailed (12).

PMM2 gene and PMM2 protein

The PMM2 gene, located on chromosome 16p13, is a small gene consisting of eight exons with a total length of approximately 6.7kb (https://www.ncbi.nlm.nih.gov/gene/5373). PMM2 is a protein consisting of 246 amino acids (11) and is widely expressed in the digestive tract, lymph nodes, adrenal glands, adipose tissue, pancreas, liver, brain tissue, and other tissues, with the highest concentrations observed in the colon and duodenum PMM2. PMM2 catalyzes the initiation of the N-glycosylation process and the second step of the mannose pathway, the isomerization of mannose-6-phosphate to mannose-1-phosphate, in the metabolism of fructose and mannose, amino and Nucleotide glycans in humans. Mannose-1-phosphate as a precursor of guanosine diphosphate (GDP-mannose), is a material required for the synthesis of polyphenol-P-oligosaccharide (Dol-P-Man), which is further involved in the synthesis of mannose and protein glycosylation (Figure 1) (13). In particular, variants in the PMM2 gene have been proved to

contribute to defects in the protein glycosylation pathway (11, 13–16), manifesting as carbohydrate-deficient glycoprotein syndrome type I (congenital disorders of glycosylation, type 1a, CDG1a), with CDG1a the most common CDG disorder. The sulfonylurea receptor (SUR) is a key protein in the regulation of insulin secretion, and it was demonstrated that glycosylation plays an important role in SUR receptor-targeted membrane transportation (17), and Cabezas et al. further revealed *in vitro* experiments that insulin secretion from pancreatic β-cells was markedly changed after SUR deglycosylation, with consequent severe effects on human metabolism.

Mutation profiles of PMM2-HI-related genes

Cumulatively, 144 mutation types were reported in PMM2 retrieved from the Human Gene Mutation Database (HGMD, www.hgmd.cf.ac.uk). As research progressed from Cabezas (5) to date, PMM2-HI has been reported in at east 18 families with more than 26 patients with PMM2-HI, and all were inherited as autosomal compound heterozygous recessive genes. At least five variant types have been reported, including four missense mutations and one splice variant (Figure 2). In each of these patients, the c.-167G>T variant, which has been proven to be a PMM2 promoter variant, leads to a decreased affinity for the PMM2 promoter ZNF143 (5), which contributes to defective glycosylation in the kidney and pancreas by altering the forming of the tridimensional structure of the chromatin ring and thus tissue-specific modulation of PMM2 enzyme transcription *in vivo*. Additionally, the ClinVar and OMIM databases were searched that the four remaining PMM2 gene variant types (c.422G>A(p.Arg141His), c.470T>C(p.Phe157Ser), c.620T>C (p.Phe207Ser), and c.255+1G>A) have all been previously described in patients with different types of CDG1a syndrome.

Genetic pathogenesis of PMM2-HI

Previous studies have shown (18) that the tridimensional structure of chromatin plays a key role in the regulation of gene expression in the coding region according to noncoding regions of genes. The loops structure, also known as topologically associated domains (TAD), is composed of chromatin and CCCTC binding factor (CTCF) on both sides, acting as the basic unit of transcriptional regulation and influences the tridimensional structure of chromatin (19, 20). The area around PMM2 contains a few CTCF sites and functional promoters. Pairs of CTCF sites interact with each other to form local chromatin loops. ZNF143 interacts with specific CTCF sites (Figure 1), and connecting CTCF with the remote regulatory elements affects the formation of chromatin loops, carrying out specific gene regulation (20–23).

Although PMM2 is generally expressed in a variety of tissues, it was demonstrated that PMM2 expression possessed certain tissue specificity (24). Hepatocyte nuclear factor 4 alpha (HNF4A), one of the transcription factors, is mainly expressed in the liver, pancreas, and kidney, and HNF4A promotes the binding of wild-type PMM2 promoter to ZNF143 during transcription to establish a functional chromatin loops loop, while the mutual interaction of mutant PMM2 promoter with ZNF143 and CTCF interactions are disrupted by HNF4A and PMM2 enzyme transcription is decreased (18, 19, 22). Thus, PMM2 promoter variant patients manifested symptoms only in the liver, pancreas, and kidneys.

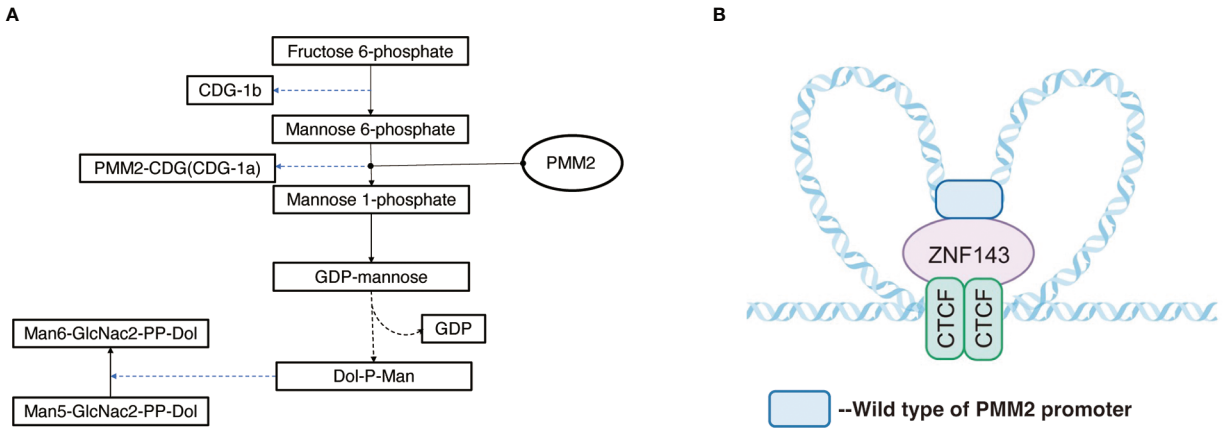

FIGURE 1
(A) Pathway of PMM2 involved. Mannose-1-phosphate is a precursor of guanosine diphosphate (GDP-mannose) which is a material required for the synthesis of polyphenol-P-oligosaccharide (Dol-P-Man) and is further involved in the synthesis of mannose and protein glycosylation.
(B) Regulation mechanism of PMM2 promoter: ZNF143 interacts with specific CTCF sites, and by connecting CTCF with the remote regulatory elements, it affects the formation of chromatin loops, carrying out specific gene regulation.

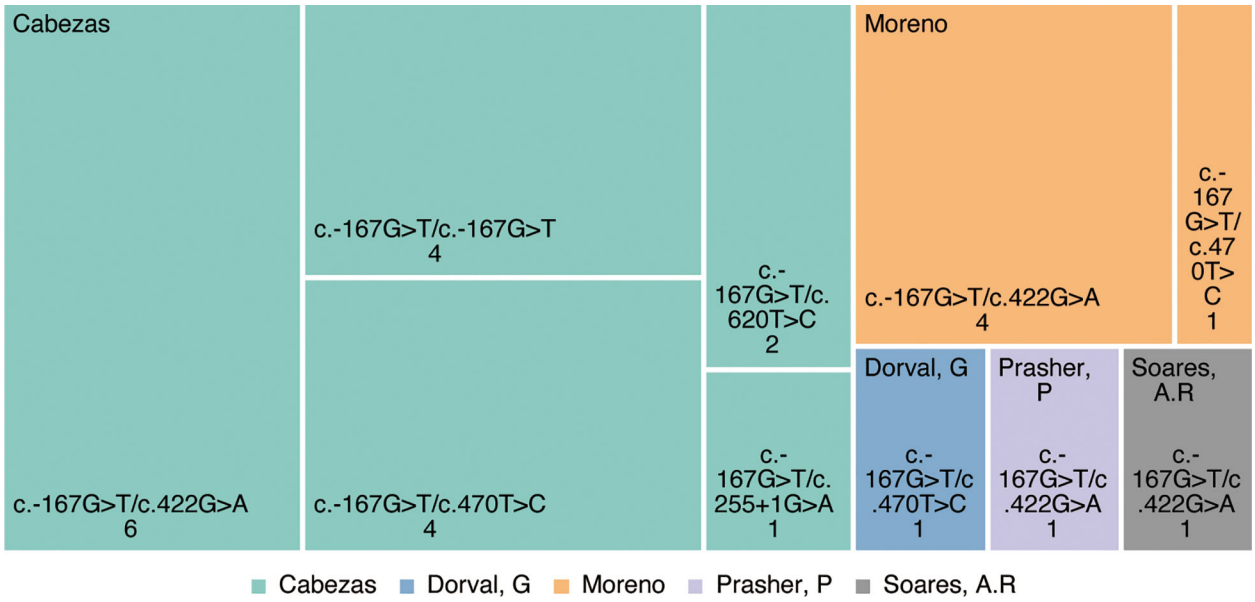

FIGURE 2
Variants in patients with PMM2-HI reported in the literature.

Decreased PMM2 enzyme expression in the liver, pancreas, and kidneys leads to impaired crucial glycosylation in their respective organs. As a consequence of impaired key protein glycosylation, it manifests in the liver as prenatal ultrasound or postnatal bile duct dilatation as well as congenital hepatic fibrosis; in the kidney it can be observed as bilateral renal enlargement, diffuse echogenic kidney, pathological examination revealing renal cysts (mainly of tubular origin) as well as huge renal cysts that resemble ARPKD. The mechanism of PMM2-HI relates to pancreatic SUR receptor deglycosylation, a key protein in the regulation of insulin secretion, and the evidence indicates (17) that glycosylation plays an essential role in SUR receptor-targeted membrane transport. SUR is a key protein in the modulation of insulin secretion. Cabeza et al. found (5) that after the stimulation of mouse β-cells with protein kinase C activator insulin secretion significantly increased after stimulation with protein kinase C activator, which demonstrates that deglycosylation exerts a significant effect on insulin secretion.

Pathological specimens of the pancreas in affected patients are also rare by the extremely rare clinical occurrence of PMM2-HI. Only two cases of pancreatic pathology in patients with PMM2-HI have been reported: one patient's pancreatic histology showed mild dilatation of the pancreatic ducts, and one patient's pancreas showed no abnormalities (10). Moreover, to the best of our acknowledgment, no 18F-L-DOPA-PET pancreatic scan results of PMM2-HI patients have been reported in the literature, which prevents definitive staging for the time being, and more research is urgently needed in the future.

Clinical characteristics of PMM2-HI

The majority of PMM2-HI patients reported to date have an autosomal recessive mode of inheritance and are from families of European ancestry. 25% probability of CHI occurring in children born to parents with PMM2-HI children and most of these patients are overweight at birth. A few cases may have onset in childhood, with the latest reported age of onset being 4 years. No association has been found between the age of onset and the severity of symptoms.

The clinical manifestations of PMM2-HI patients are primarily polycystic kidneys, hyperinsulinemic hypoglycemia, and polycystic liver. Bilateral renal enlargement, diffuse echogenic kidneys, and diminished hepatic parenchymal differentiation, and multiple small cysts around the liver may be detected on prenatal ultrasound or after birth. Chronic kidney disease symptoms progress slowly, especially after the age of 35 years when the progression of kidney disease is further slowed.

Patients with hypoglycemia mostly onset with epilepsy, without the manifestation of high blood ammonia and liver function abnormalities, occasionally with high serum lactate. Compared with CDG1a patients with PMM2 gene variant, PMM2-HI patients showed no neurological impairment and no transferrin hydroelectric focusing. However, in a case reported by Dorval et al. (10) fetal ultrasonography showed abnormally enlarged kidneys at 23 weeks of the first pregnancy in an unrelated couple. After the termination of pregnancy at 25 weeks, neuropathological examination showed cerebellar hypoplasia with the denticulate nucleus and optic nerve

defects, presumably associated with the patient's simultaneous heterozygosity for CDG1a-related variants.

Diagnosis of PMM2-HI

PMM2-HI is diagnosed in the same manner as other types of CHI, based on the child's presentation of non-ketotic hypoglycemia, requiring large amounts of glucose infusion to control hypoglycemic episodes, and overproduction of insulin which is incompatible with hypoglycemia. According to the guidelines published by Ferrara et al. (25) in 2016, the diagnostic criteria for CHI are as follows: when intravenous plasma glucose <2.8 mmol/L accompanied by asynchronous insulin secretion (usually >1-2 μU/ml; or still detectable C-peptide >0.2 mmol/L), low levels of β-hydroxybutyric acid (<1.8 mmol/L), low free fatty acids (<1.7 mmol/L), positive glucagon stimulation test (test procedure: 1 mg glucagon intramuscularly or intravenously, a neonatal dose of 0.5 mg, blood glucose elevation ≥1.7 mmol/L). Besides the above indicators, PMM2-HI can also be diagnosed by genetic examination with the genetic variant c.-167G>T, which is typical differential diagnostic evidence of PMM2-HI.

Treatment of PMM2-HI

The treatment of PMM2-HI patients in the acute stage is consistent with those of other types of CHI patients, in which rapid infusion of glucose (26) (reported in the literature (6) at a maximum rate of up to 17.6 mg/kg/min) can be infused to prevent and mitigate the onset of hypoglycemia in order to avoid permanent damaging of the neurological system of the child due to hypoglycemic episodes.

Pharmacologically, diazoxide is the first choice for the control of hypoglycemic episodes in CHI patients, and it is a KATP channel activator (27–29). Since PMM2 promoter gene variants did not induce structural changes in KATP channels, about 54% (14/26) of patients received diazoxide treatment, and it was shown that all PMM2-HI patients responded effectively to diazoxide treatment. The most frequent complications of diazoxide are sodium retention and pulmonary hypertension (30, 31). In children who are at risk of water and sodium retention and pulmonary hypertension and require high volumes of glucose infusion to control the occurrence of hypoglycemia, a thiazide diuretic (hydrochlorothiazide 1-2mg/kg/day, bid) can be used in advance to prevent heart failure (32). Other side effects of diazoxide include neutropenia, blood volume hypertension, and hypertrichosis. There is no literature reported on the application of octreotide to treat patients with PMM2-HI.

Using captopril (0.2 mg/day) is also recommended to improve renal function in patients with decreased GFR due to polycystic kidneys.

To date, there is no literature reporting the following treatments for PMM2-HI: hormone therapy (such as glucagon) and surgical therapy (focal lesion resection or diffuse lesion near-total pancreatectomy), which are commonly used as treatment strategies for patients with CHI besides pharmacological treatment.

Prognosis of PMM2-HI

As reported in the available studies (5–10, 12), patients with PMM2-HI treated with diazoxide are clinically effective with diazoxide medication, hence, if the diagnosis and reasonable treatment are performed early, complications such as neurological damage due to persistent recurrent hypoglycemia can be effectively avoided and the clinical prognosis is generally promising. There is no report of spontaneous recovery in children with PMM2-HI.

The majority of children with PMM2-HI have an early onset of symptoms and are most effective on diazoxide therapy. In clinical practice, children with prenatal ultrasound diagnosis of polycystic kidney for which attention should be paid to monitor their blood glucose and insulin levels. Once the child's blood glucose has recovered to normal levels, follow-up testing of the child's kidney and liver function should be performed. Genetic tests such as sanger should be performed promptly in children with diagnosed CHI which can assist in genetic typing, formulation of a more effective and precise therapeutic strategy, and effectively improve the prognosis of children with CHI (33–35).

Meanwhile, more basic research and clinical research reports on the mechanism of PMM2 promoter variants are needed in the future to further clarify the type of pancreatic pathology, neurological damage symptoms, and therapeutic options for PMM2-HI.

Author contributions

CC wrote the manuscript and performed the research in the medical literature. YS reviewed the manuscript. All the authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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References

1. Giri D, Hawton K, Senniappan S. Congenital hyperinsulinism: Recent updates on molecular mechanisms, diagnosis and management. *J Pediatr Endocrinol Metab* (2022) 35(3):279–96. doi: doi: 10.1515/jpem-2021-0369

2. 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

3. 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

4. Müller D, Zimmering M, Roehr CC. Should nifedipine be used to counter low blood sugar levels in children with persistent hyperinsulinaemic hypoglycaemia? *Arch Dis Child* (2004) 89(1):83–5. doi: 10.1136/fn.89.1.f83

5. 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

6. Prasher P, Redmond K, Stone H, Bailes J, Nehus E, Preston D, et al. Persistent hypoglycemia with polycystic kidneys: A rare combination - a case report. *BioMed Hub.* (2020) 5(3):32–7. doi: 10.1159/000511389

7. Moreno Macián F, De Mingo Alemany C, León Cariñena S, Ortega López P, Rausell Felix D, Aparisi Navarro M, et al. Mutations in PMM2 gene in four unrelated Spanish families with polycystic kidney disease and hyperinsulinemic hypoglycemia. *J Pediatr Endocrinol Metab* (2020) 33(10):1283–8. doi: 10.1515/jpem-2020-0168

8. 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

9. Islam S, Tekman M, Flanagan SE, Guay-Woodford L, Hussain K, Ellard S, et al. Founder mutation in the PMM2 promotor causes hyperinsulinemic hypoglycaemia/polycystic kidney disease (HIPKD). *Mol Genet Genomic Med* (2021) 9(12):e1674. doi: 10.1002/mgg3.1674

10. Dorval G, Jeanpierre C, Morinière V, Tournant C, Bessières B, Attié-Bittach T, et al. Cystic kidney diseases associated with mutations in phosphomannomutase 2 promotor: a large spectrum of phenotypes. *Pediatr Nephrol.* (2021) 36(8):2361–9. doi: 10.1007/s00467-021-04953-9

11. Matthijs G, Schollen E, Pardon E, Veiga-Da-Cunha M, Jaeken J, Cassiman JJ, et al. Mutations in PMM2, a phosphomannomutase gene on chromosome 16p13, in carbohydrate-deficient glycoprotein type I syndrome (Jaeken syndrome). *Nat Genet* (1997) 16(1):88–92. doi: 10.1038/ng0597-88

12. Vuralli D, Yildiz Y, Ozon A, Dursun A, Gonc N, Tokatlı A, et al. 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

13. Matthijs G, Schollen E, Bjursell C, Erlandson A, Freeze H, Imtiaz F, et al. Mutations in PMM2 that cause congenital disorders of glycosylation, type ia (CDG-ia). *Hum Mutat* (2000) 16(5):386–94. doi: 10.1002/1098-1004(200011)16:5<386::AID-HUMU2>3.0.CO;2-Y

14. Lam C, Krasnewich DM, et al. PMM2-CDG. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, editors. *GeneReviews®*. Seattle (WA: University of Washington, Seattle (1993).

15. Vaes L, Rymen D, Cassiman D, Ligezka A, Vanhoutvin N, Quelhas D, et al. Genotype-phenotype correlations in PMM2-CDG. *Genes (Basel)* (2021) 12 (11):1658. doi: 10.3390/genes12111658

16. Wolthuis DF, van Asbeck EV, Kozicz T, Morava E. Abnormal fat distribution in PMM2-CDG. *Mol Genet Metab* (2013) 110(3):411–3. doi: 10.1016/j.ymgme.2013.08.017

17. 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

18. Dekker J, Mirny L. The 3D genome as moderator of chromosomal communication. *Cell* (2016) 164(6):1110–21. doi: 10.1016/j.cell.2016.02.007

19. Dixon JR, Selvaraj S, Yue F, Kim A, Li Y, Shen Y, et al. Topological domains in mammalian genomes identified by analysis of chromatin interactions. *Nature* (2012) 485(7398):376–80. doi: 10.1038/nature11082

20. Bailey SD, Zhang X, Desai K, Aid M, Corradin O, Cowper-Sal Lari R, et al. ZNF143 provides sequence specificity to secure chromatin interactions at gene promoters. *Nat Commun* (2015) 2:6186. doi: 10.1038/ncomms7186

21. Grossman CE, Qian Y, Banki K, Perl A. ZNF143 mediates basal and tissue-specific expression of human transaldolase. *J Biol Chem* (2004) 279(13):12190–205. doi: 10.1074/jbc.M307039200

22. Wen Z, Huang ZT, Zhang R, Peng C. ZNF143 is a regulator of chromatin loop. *Cell Biol Toxicol* (2018) 34(6):471–8. doi: 10.1007/s10565-018-9443-z

23. Carney EF. Polycystic kidney disease: PMM2 mutation causes PKD and hyperinsulinism. *Nat Rev Nephrol.* (2017) 13(6):321. doi: 10.1038/nrneph.2017.58

24. Grünewald S, Schollen E, Van Schaftingen E, Jaeken J, Matthijs G. High residual activity of PMM2 in patients' fibroblasts: possible pitfall in the diagnosis of CDG-ia (phosphomannomutase deficiency). *Am J Hum Genet* (2001) 68(2):347–54. doi: 10.1086/318199

25. 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

26. 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(1):e246–e53. doi: 10.1210/clinem/dgab601

27. Kane C, Lindley KJ, Johnson PR, James RF, Milla PJ, Aynsley-Green A, et al. Therapy for persistent hyperinsulinemic hypoglycemia of infancy. understanding the responsiveness of beta cells to diazoxide and somatostatin. *J Clin Invest.* (1997) 100(7):1888–93. doi: 10.1172/JCI119718

28. Shyng S, Nichols CG. Octameric stoichiometry of the KATP channel complex. *J Gen Physiol* (1997) 110(6):655–64. doi: 10.1085/jgp.110.6.655

29. 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

30. 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

31. Herrera A, Vajravelu ME, Givler S, Mitteer L, Avitabile CM, Lord K, et al. Prevalence of adverse events in children with congenital hyperinsulinism treated with diazoxide. *J Clin Endocrinol Metab* (2018) 103(12):4365–72. doi: 10.1210/jc.2018-01613

32. 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

33. 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(1):9–21. doi: 10.1111/dme.13823

34. 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

35. Galcheva S, Al-Khawaga S, Hussain K. Diagnosis and management of hyperinsulinaemic hypoglycaemia. *Best Pract Res Clin Endocrinol Metab* (2018) 32(4):551–73. doi: 10.1016/j.beem.2018.05.014

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Continuous glucose monitoring for children with hypoglycaemia: Evidence in 2023

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In 2023, childhood hypoglycaemia remains a major public health problem and significant risk factor for consequent adverse neurodevelopment. Irrespective of the underlying cause, key elements of clinical management include the detection, prediction and prevention of episodes of hypoglycaemia. These tasks are increasingly served by Continuous Glucose Monitoring (CGM) devices that measure subcutaneous glucose at near-continuous frequency. While the use of CGM in type 1 diabetes is well established, the evidence for widespread use in rare hypoglycaemia disorders is less than convincing. However, in the few years since our last review there have been multiple developments and increased user feedback, requiring a review of clinical application. Despite advances in device technology, point accuracy of CGM remains low for children with non-diabetes hypoglycaemia. Simple provision of CGM devices has not replicated the efficacy seen in those with diabetes and is yet to show benefit. Machine learning techniques for hypoglycaemia prevention have so far failed to demonstrate sufficient prediction accuracy for real world use even in those with diabetes. Furthermore, access to CGM globally is restricted by costs kept high by the commercially-driven speed of technical innovation. Nonetheless, the ability of CGM to digitally phenotype disease groups has led to a better understanding of natural history of disease, facilitated diagnoses and informed changes in clinical management. Large CGM datasets have prompted re-evaluation of hypoglycaemia incidence and facilitated improved trial design. Importantly, an individualised approach and focus on the behavioural determinants of hypoglycaemia has led to real world reduction in hypoglycaemia. In this state of the art review, we critically analyse the updated evidence for use of CGM in non-diabetic childhood hypoglycaemia disorders since 2020 and provide suggestions for qualified use.

KEYWORDS

hypoglycaemia, continuous glucose monitoring, children, hyperinsulinism, glycogen storage disease, prematurity

1 Introduction

In 2023, non-diabetes hypoglycaemia remains a major global problem for children. Its effects are far reaching, with impacts on quality of life (1, 2), health economics (3), hypoglycaemia fear (4), reaching beyond the individual to the extended family (5, 6). Although recent studies (7), complimenting previous work (8, 9), have suggested a lesser effect of transient neonatal hypoglycaemia (10), there remains little doubt of the impact of severe childhood hypoglycaemia on neurodevelopmental delay, particularly in those children with severe and recurrent hypoglycaemia due to congenital hyperinsulinism (CHI) (9–11).

Essential to all hypoglycaemia management, irrespective of the cause, is the detection, prediction and prevention of episodes through glucose testing (12, 13). The first of these three tasks has been traditionally performed by fingerprick blood glucose testing (13), with prediction and prevention reliant on clinical skill and patient experience. However, over recent years, all three tasks are increasingly being performed by continuous glucose monitoring (CGM) in either its raw form or through its manipulation by modern computer algorithmics. For people living with diabetes, CGM and associated predictive algorithms are widely used and well established in the reduction of hypoglycaemia (14–17) and cost-effectiveness (18–20). However, for those with a non-diabetes hypoglycaemia disorder, the utility in diabetes has not been replicated and CGM has not been established in routine clinical practice.

The use of CGM in rare hypoglycaemia disorders is a rapidly evolving and expanding field. In this review we have followed on from a comprehensive review in 2020 (13), to provide an update on improvements in the technology and utility of CGM focusing mainly on CHI, glycogen storage diseases (GSD) and neonatal prematurity. We reflect on our predictions from 2020, synthesise current understanding and look to the future.

2 Accuracy

We have detailed the background to accuracy assessments in CGM elsewhere (13) but it is worth outlining the two differing approaches to accuracy assessment: 1) pairing CGM values with fingerprick glucometer values and measuring difference; 2) evaluating the ability of CGM devices to 'detect' hypo(or hyper)glycaemia within a time window and thus utilising to a fuller extent the semi-continuous nature of CGM. Measures of accuracy differ widely throughout the literature, but the former is more commonly used and tends to incorporate mean absolute relative difference (MARD), mean absolute difference and hypoglycaemia sensitivity/specificity. A summary of CGM accuracy studies by various groups using different CGM devices in non-diabetes hypoglycaemia is presented in Table 1.

2.1 Neonates

Beardsall et al. first evaluated the accuracy of CGM devices in neonates in 2005 (21) and later in 2013 (22); they reported a correlation coefficient of 0.69-0.94 with safe results on an error grid (albeit one designed for those with diabetes). However, hypoglycaemia sensitivity was found to be only 17%. More recent results from the same group showed a relatively small MARD of 11% but a hypoglycaemia sensitivity of only 59% with the latest devices and technologies (23). These calculations were based on a lower threshold for hypoglycaemia (<2.6mmol/l) than is usually used outside the neonatal unit. Furthermore, as described above, sensitivity is based on point comparisons of accuracy which can underestimate the clinical value of sensor glucose trends in detecting hypoglycaemic events. Recent work in Australia by Vijayanand et al. (24) has confirmed the poor hypoglycaemia sensitivity seen in this group with results of 54% when using point comparisons.

TABLE 1 Accuracy data for CGM use in non-diabetes childhood hypoglycaemia disorders.

Publication	Patient group	Device	MARD (%)	MD (mmol/L)	MAD (mmol/L)	R^2	Hypo sensitivity
Beardsall '05(21)	Neonates	Medtronic MiniMed	—	-0.1	—	0.87	N/A
Beardsall '13(22)	Neonates	Medtronic System Gold	—	—	—	0.94	(2.6mmol/L) 17%
Win(23)	Neonates (+/- CHI)	Medtronic Paradigm OR Dexcom G4	11.0	—	—	—	(2.6mmol/L) 59%
Vijayanand(24)	Neonates (+/- CHI)	Dexcom G4	13.1	+0.3	—	—	(3.5mmol/L) 78% (3.0mmol/L) 54%
Alsaffar(25)	CHI	Abbott Freestyle Libre	17.9	+0.3	—	0.70	(3.5mmol/L) 52%
Rayannavar(26)	CHI	Dexcom G5	17.5	-0.4	—	—	(3.9mmol/L) 86% (3.0mmol/L) 66%
Worth(27)	CHI	Dexcom G6	19.3	+0.4	0.9	—	(3.9mmol/L) 52% (3.5mmol/L) 45% (3.0mmol/L) 40%
Kasapkara(28)	GSD	Medtronic	—	—	—	0.74	—
Herbert(29)	GSD	Dexcom G4	—	—	—	0.57	—
Rossi(30)	GSD	Dexcom G6	—	+0.9	—	—	—

MARD, mean absolute relative difference; MD, mean difference; MAD, mean absolute difference; R^2, correlation coefficient between blood glucose and CGM glucose levels; Hypo, hypoglycaemia.

2.2 Childhood hypoglycaemia disorders

CGM is not routinely used in patients with CHI and therefore data is relatively sparse (Table 1). In the first evaluation of CGM in CHI, Alsaffar et al. (25) reported a hypoglycaemia (3.5mmol/L) sensitivity of only 52% but did not report a MARD. While an evaluation of a more up to date device by Rayannavar et al. (26) showed a better hypoglycaemia sensitivity of 86%, this was calculated using a higher cut-off for hypoglycaemia (3.9mmol/L), as is standard practice in some countries. When hypoglycaemia <3.0mmol/L was investigated, a low sensitivity of 66% was demonstrated. As existing error grids (such as Parks and Clarke) are designed for evaluation of CGM accuracy for those with diabetes, they have not been used as standard in assessments in CHI. Recently Worth et al. (27) developed an expert-consensus error grid for use in CHI and used this to evaluate the accuracy of one of the most recent CGM sensors, the Dexcom G6. Results suggested the presence of significant clinical risk in the use of CGM for patients with CHI due to poor device accuracy on error grid analysis and hypoglycaemia sensitivity of only 45%. Analysis of the ability of the Dexcom G6 to detect glucometer-measured hypoglycaemia within a 30 minute window was marginally better but still unreliable at 51% (27).

Equally, CGM is also not used routinely in patients with GSD and assessments of CGM accuracy for this group have been largely incomplete (Table 1). These demonstrate correlation between CGM and glucometer values but the magnitude of error has not been reported. Papers (28, 29) report mean difference or correlation but due to the presence of both overestimation and underestimation, and no report of mean *absolute* difference, it is impossible to determine the average magnitude of errors. Rossi et al. (30) went on to evaluate CGM error by glucose value and also between those with GSD1a and healthy volunteers. They found that CGM overestimation was worse for those with GSD1a and at glucose values <3.9mmol/L, thereby increasing the risk of missed hypoglycaemia for the most vulnerable groups at the time of greatest need.

3 Efficacy of CGM to detect and prevent hypoglycaemia

We have previously summarised the efficacy of CGM for children with non-diabetes hypoglycaemia due to various conditions (13). Here we summarise recent developments in the field with regards to the conventional use of CGM to detect and prevent hypoglycaemia by simple provision to patients and clinicians. The non-conventional use of CGM is discussed later in Section 7.

3.1 Neonates

Previously summarised studies (13) have demonstrated the utility of CGM to reduce painful procedures, detect unsuspected hypoglycaemia and reduce *hyper*glycaemia. More recently, Fernández Martínez et al. (31) confirmed the ability of CGM to detect unsuspected and prolonged hypoglycaemia in very low birth weight (VLBW) neonates. Win et al. (23) have since demonstrated significant fluctuations in glucose in neonates; more pronounced in those with CHI. The same group recently published the results of an international, multi-centre RCT investigating the use of CGM in preterm neonates and clearly demonstrated a reduction in hypoglycaemia and hyperglycaemia for those in the CGM group (32) encouraging CGM as a potential tool for regular use in the neonatal intensive care unit.

3.2 Hypoglycaemia associated with rare endocrine conditions

At the time of our previous review in 2020, there was no evidence for CGM reducing hypoglycaemia for children with any endocrine conditions other than diabetes mellitus. In the absence of larger scale studies, we discussed (13) minimal evidence for use of CGM for both adults and children with adrenal insufficiency (AI) and the anecdotal reports of CGM use for those with CHI.

Further single-case, anecdotal reports of utility of CGM in CHI (33) and hypopituitarism (34) have since been published. Importantly however, Worth et al. have recently published non-randomised data on CHI patients with periods of blinded and unblinded CGM (35); suggesting that the simple provision of CGM (without expert or algorithmic interpretative support) does *not* reduce hypoglycaemia for those with CHI. The addition of interpretative algorithmic or clinical support is discussed in Section 7. However, at the time of writing, there are no comprehensive studies evaluating the efficacy of CGM to reduce hypoglycaemia for children with endocrine hypoglycaemia.

3.3 Hypoglycaemia associated with rare hereditary metabolic disorders

We have previously outlined (13) the utility of CGM to detect unsuspected hypoglycaemia and facilitate manipulation of diet and treatment for patients with GSD. Previous anecdotal reports highlighted the utility of retrospective CGM data analysis but advised against the provision of real-time CGM to patients for fear of inappropriate treatment alterations (36). Since our previous review, there have been further anecdotal reports of CGM utility in the detection of glycaemic variability and excursions for patients with metabolic causes of hypoglycaemia (37–39) but no systematic evaluations of the use of CGM to actually prevent or reduce hypoglycaemia.

4 Family perspectives

Our previous review discussed families with CHI and GSD reporting marginal benefit from the use of CGM as secondary outcomes of studies. Anecdotally, families found glucose trends helpful. Since 2020, the significant increase in the use of CGM in hypoglycaemia disorders has led to an increase in literature regarding families' perceptions of this emerging technology, described below.

4.1 Patient charity reports

Patient charities fulfil a vital role of providing support to those with hypoglycaemia conditions but also provide an important window into the views and opinions of families. In a recent unpublished study (summarised in an opinion paper (40)), the UK Children's Hyperinsulinism Charity (UK CHC) reported that families with CHI find CGM: offers a safety net, improves quality of life, and reduces worry. Patients reported (40) difficulty in access to CGM and a call was made for wider availability for families with CHI. While this survey is likely subject to significant positive sampling bias, it does offer an important insight into the opinions of some families with CHI.

The charity Congenital Hyperinsulinism International (CHI) recently revealed that 45.7% of respondents to a global registry use CGM but that access to devices is often a problem and trust in the data generated is often low (2). They also report that families generally find devices useful but that patients experience problems with poor accuracy (6). Again, this is likely open to sampling bias but offers an important user-perspective. Within GSD, CGM is a much higher research priority for healthcare professionals than it is for patients and carers who rank it as a lower priority (41).

4.2 Qualitative studies

While patient organisations have called for wider access to CGM, it is important to formally assess families' experiences of CGM to actively seek out both positive and negative views. As recently highlighted by Peeks et al. (42), "glucose management as assessed with CGM should be balanced against psychosocial well-being and quality of life" which cannot be assumed to be higher with CGM than without.

In CHI patients, Auckburally et al. (43) undertook semi-structured interviews with families who had been provided with a CGM for 12 weeks as part of a research project. As there was no existing information on CHI families' experiences of CGM, the authors performed a thematic analysis to identify themes important to patients and their families. Such detailed analysis revealed a rich and complex mixture of attitudes towards CGM. Families reported positive feelings about CGM's function as an educational tool which could motivate behavioural changes to prevent hypoglycaemia. However, the problematic issues of poor accuracy and irritating alarms were raised by all participants.

In order to better understand the reasons for a high rate of dissatisfaction with CGM seen in CHI families, Ahmad et al. (44) performed semi-structured telephone interviews with those who had discontinued use. Primary reasons for discontinuation were pain, device inaccuracy, issues with technical setup and 90% of those surveyed thought that CGM device use would have been easier if their child had been a different age (either younger or older) (44). Comprehensive assessments of families' experiences of CGM, with a focus on the reduction of selection bias, are essential in the journey to establish CGM as a therapeutic option for paediatric hypoglycaemia disorders. The authors are aware of two separate studies aiming to achieve this for families with CHI and the results are eagerly awaited.

5 Barriers to the use of CGM

In our 2020 review we highlighted the barriers to wider use of CGM in paediatric hypoglycaemia disorders and to date there are no improvements with regards to lag time, alarms or fingerprick testing. However, with regards to clinician inertia and cost, an update is worthwhile.

5.1 Clinician inertia and usability

Over the last three years, the authors have noticed a significant increase in the interest in CGM by clinicians working in paediatric hypoglycaemia disorders. There is now less suspicion of the technology and a higher acceptance of using CGM as a routine part of care. This is mirrored in the significant increase in publications relating to CGM in both hypoglycaemia disorders and neonatology. However, the interest and marketing strategy of device manufacturers remains firmly focused on diabetes mellitus, precluding wider adoption and development specific to hypoglycaemia.

5.2 Cost and widening access

As CGM technology develops, it is important that the availability of devices is considered, especially for those in low-income countries (LICs) and for patients with rare diseases. These groups are often marginalised and disadvantaged in the commercially-driven push for technological progression but efforts must be made to minimise access inequalities (45). As a technology, CGM could arguably have significant impact in LICs due to the added burden of hypoglycaemia from malaria, malnutrition, diarrhoea and sepsis (46). Additionally, for people living with diabetes, access to insulin is often intermittent in LICs (47), leading to hypoglycaemia and hyperglycaemia. CGM would also be highly valuable in the neonatal setting as capacity for regular glucose monitoring in neonatal units in LICs is often limited and neonatal mortality is high (48). Indeed, neonatal hypoglycaemia is often present in otherwise uncomplicated newborn infants, and recognition and treatment may have a significant impact on neonatal outcomes (49, 50).

Moreover, the long-term impacts associated with childhood hypoglycaemia, such as neurodisability, epilepsy and reduced cognitive function (9, 51) have a higher burden in LICs, being poorly understood by wider society and suboptimally managed due to meagre resources (52–54). So, while the costs of CGM may be high, its implementation may enable faster, accurate treatment modification, improving outcomes (38) and likely contributing to value based healthcare in both common, high volume disease (55) and rare, low volume disease such as GSD (56). However, it is important to recognise that technology developed for a high-income setting is not always appropriate for LICs where the environment is different; there can be extremes of temperatures, intermittent access to internet and electricity, high levels of dust and minimal access to engineers to repair devices (50, 57–59). A target product profile (TPP)-based approach has been developed to identify key specifications for product innovation in LICs. This approach has been particularly

successful in development of neonatal devices, most notably in bubble CPAP, and a similar approach should be considered in the development of CGM devices (50, 60).

6 Updates on previously suggested developments

In our 2020 review we predicted that future developments would be focused on CGM device technology and predictive hypoglycaemia algorithms. Here we provide an update on the developments in these areas over the last three years before moving on to discuss alternative and novel areas for CGM use in Section 7.

6.1 CGM device technology

The direction for CGM device technology development continues towards miniaturisation, with a focus on reducing the invasive nature of some CGM devices. Dexcom® have since released the G7 device which is smaller, thinner and predicted to be more accurate. Abbott® have released the Freestyle Libre 3, also smaller and thinner and now offering real time readings with optional alerts. Eversense® now have an implantable sensor with a six month wear time and requiring only a single calibration per day.

There has also been significant interest in the last few years on optical sensors that detect photons to determine the glucose concentration via the interaction between glucose molecules and different wavelengths of light (61). Other sensor developments focus on the non-invasive measurement of sweat, urine, saliva, tears (62) and even thermal monitoring (63); however, these ideas have not yet translated to a commercially viable stage.

6.2 Predictive hypoglycaemia algorithms

Our 2020 review (13) outlined the background to the use of predictive algorithms for hypoglycaemia and the different forms that these can take; physiological, data-driven, and hybrid (64). While non-machine learning algorithms such as Model Predictive Control have been beneficial for adults (65) and neonates (66) using closed loop insulin delivery, these systems are of no use to the majority of patients with rare hypoglycaemia disorders whose hypoglycaemia is not caused by exogenous insulin. Work in the field of data-driven predictions continues to expand rapidly in diabetes and artificial intelligence and machine learning methods using large historical datasets continue to be used to derive theoretical prediction models (Figure 1). While, multiple groups have continued to publish increasingly accurate in-silico algorithms (67–70), these have been evaluated by systematic review (71) and meta-analysis (72) and found to have insufficient ability to detect and prevent hypoglycaemia. The authors conclude that improvement is required before application in clinical settings. As suggested, these algorithms have been evaluated in-silico only with no conclusive examples of Machine Learning-driven predictive algorithms reducing hypoglycaemia in the real world.

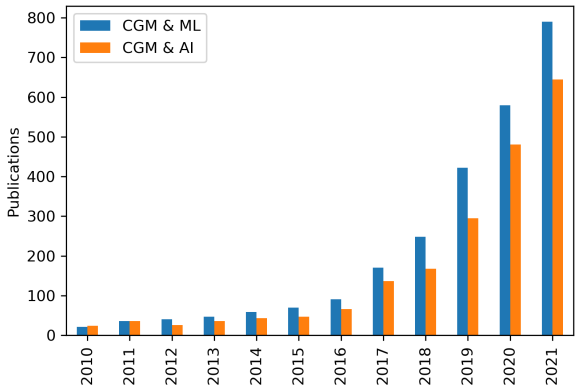

FIGURE 1
Publications by year with the search terms "continuous glucose monitoring" (CGM) and "machine learning" (ML) or "artificial intelligence" (AI), found on Google Scholar.

Decision Support Systems (DSS) are an extension of glucose predictive algorithms and facilitate decision making (e.g. food intake) based on various inputs (e.g. CGM data) and predicted outcomes (e.g. hypoglycaemia). Recent DSSs have shown in-silico (73) and possibly real world (74) reduction in hypoglycaemia through modification of insulin dosing for people living with diabetes. However, Tyler et al. (75) note in their systematic review that "it has not yet been shown that a DSS can improve time in range in human studies" and more work is required. Vitally, all DSSs focus on the use of exogenous insulin as either an input or output and are therefore of no use to those with a rare hypoglycaemia disorder such as CHI or GSD but may have potential in neonates on insulin therapy (66).

7 Novel directions and a possible future for CGM in hypoglycaemia

So far we have provided updates on areas covered in our previous review. In this section we move on to discuss novel areas and uses for CGM which have either emerged since 2020 or are now gaining prominence. Person-centred outcome measures have been defined for type 1 diabetes (76, 77) but are currently lacking for rare hypoglycaemia disorders. This causes difficulty in comparing studies and evaluating day to day impact for patients. Consensus, person-centred outcomes would greatly enhance routine healthcare and research for these groups, particularly with regards to emerging but as yet unproven technologies such as CGM.

7.1 CGM to elicit patterns and digital phenotypes

There is increasing recognition of phenotypes beyond those classically described by physical traits or cellular changes. Most recently established is the "digital phenotype" (78). The digital phenotype covers both aspects of behaviours related to technology such as social media use use as well as behaviours measured by

technology such as heart rate monitors, accelerometers and CGM. These new measures facilitate a more comprehensive and individualised picture of patients' health and contribute to "P4 medicine" (79); allowing for a predictive, preventative, personalised and participatory approach to management.

Worth et al. (80) took the first steps towards extending the digital phenotype of CHI with their analysis of retrospectively collected CGM data. Previously collected CGM data was used to identify periods of high hypoglycaemia risk in the early morning in patients with CHI; opening the door for targeted interventions on a group and individual level. Further work by this group (81) investigated patterns of hypoglycaemia at an individual level and found that each patient with CHI had clear and individual *weekly* patterns for repeated hypoglycaemia. Peeks et al. (42) performed a similar analysis in patients with hepatic GSD to provide the first insight into CGM profiles in this patient group and similarly concluded that analysis on a group level was of some use but improved when performed on an individual basis.

Further contributions to the digital phenotypes of hypoglycaemia disorders have been made by Rossi et al. (30) who provided CGM metrics for glycaemic variation and control in adult patients with GSD1a and compared this to healthy volunteers. Worth et al. (82) performed a similar analysis for patients with CHI on a larger scale (3.4 million data points) but without healthy controls to establish a national baseline of hypoglycaemia and confirm earlier reports (80) of daily hypoglycaemia patterns at a group level. Finally, Park et al. (83) recently reported preliminary data from the GRACE trial, establishing the extent of glucose variability in children with adrenal insufficiency compared to healthy controls.

7.2 CGM as a behaviour change tool

CGM is still in its infancy as a technology and new ways are being explored to derive positive impact for patients' health. Traditional usage has focused on high frequency glucose data to allow patients to adjust insulin doses and to predict upcoming excursions from euglycaemia. As discussed above, CGM has been adopted by the computer science community with a focus on the development of glucose forecasting algorithms (64, 84) to improve the accuracy with which these excursions are predicted.

However, a new direction for CGM use is now being investigated, CGM as a behaviour change tool. In their review, Ehrhardt and Zaghal (85) conclude that "Rather than being used as a "reactionary device" for hypoglycaemia prevention and glycaemic management, CGM should be assessed for its use as a prevention tool. Its potential role as an adjunct to lifestyle changes […] calls for further evaluation". In a survey of 40 people living with diabetes (86), 90% commented that CGM contributed to a healthier lifestyle, with 87% modifying food choices and 47% increasing physical activity based on CGM. Recent publications have also suggested that CGM could act as a behaviour modification tool for those with obesity (87).

Combining pattern recognition with behaviour change has the potential to significantly improve self-management behaviours (88). Worth et al. used CGM to identify individual patterns in weekly hypoglycaemia risk of patients with CHI (81). The same group developed interpretative algorithms to facilitate patient understanding of patterns and provided suggestions for reflection designed to modify parental behaviours (35). The resulting change in fingerprick and self-management behaviours led to a reduction in real world hypoglycaemia of 25% (35, 81), demonstrating the potential power of using CGM as a tool to identify and modify the behavioural determinants of hypoglycaemia. Due to the focus on weekly patterns and behavioural determinants of hypoglycaemia, this approach is less subject to problems with poor point accuracy and patient dissatisfaction with alarms, suggesting a novel and sustainable path to CGM application.

7.3 CGM to diagnose and inform management

While children with rare hypoglycaemia disorders do not have exogenous insulin to adjust based on CGM readings, there are many other diagnostic and management decisions that can be made upon the basis of CGM outputs. Work evaluating the CGM profiles of healthy subjects (89, 90) provides more data with which researchers can compare results from disease cohorts and evaluate glycaemic control in context. Rossi et al. (30) have shown this with their own assessment of healthy subjects in comparison to those with GSDIa. Separately, Rossi et al. (91) propose the use of CGM in a hybrid approach to determine fasting tolerance in children with GSDs rather than the traditional "controlled fast" with multiple fingerprick tests. They go on to highlight the efficacy of CGM to determine incidence of nocturnal hypoglycaemia as well as the impact of diet and medications on glycaemic profiles. Peeks et al. (42) support this approach and have documented their use of CGM to monitor the impact of nocturnal dietary interventions, changes in starch loads, and treatment with empagliflozin for patients with hepatic GSDs. In the case of treatment with empagliflozin, the authors highlight the utility of CGM to detect the potential hypoglycaemia resulting from medication-induced glycosuria (42). Logel et al. (92) similarly used intermittent CGM to initiate and then titrate doses of diazoxide in a patient with Glut1 deficiency who had failed ketogenic diet; without the high granularity data of CGM it was felt that diazoxide would have been administered at incorrect doses, risking the loss of efficacy seen in other cases treated without CGM.

7.4 CGM as an outcome marker in clinical trials

In recent years CGM has become popular as an outcome in clinical trials to determine efficacy of interventions to reduce hypoglycaemia. The high granularity data generated by CGM reduces the chance of type II errors in clinical trials and allows investigators better insight into glycaemic changes secondary to therapeutics.

CGM has recently been used as an outcome measure for: hypoglycaemia after paediatric cardiac surgery (93); treatment of CHI with Dasiglucagon (94); treatment of CHI with RZ358 (95); treatment of GSDIa with AAV8 gene transfer (96) and is planned for more upcoming therapeutic trials in rare hypoglycaemia disorders. An essential component of using CGM as an outcome measure is

understanding the baseline data for each disease and population (42). This requires quantification of as many patients as possible (79); Rossi et al. (30) recently provided the first publication of CGM metrics for patients with GSD1a, as did Worth et al. (80, 82) for patients with CHI, essential datasets for those utilising baseline characteristics when designing future therapeutic trials using CGM for primary or secondary outcomes.

8 Conclusion

There has been considerable progress in the development of the relatively new technology of CGM. However, in childhood hypoglycaemia disorders many historical problems remain. CGM continues to be insufficiently accurate, somewhat burdensome for patients and their families, costly, and lacking in evidence for its ability to reduce hypoglycaemia when provided to families without support. However, there is scope for optimism. Devices continue to miniaturise, improve in accuracy and reduce patient burden. Research and clinical teams are working around suboptimal point accuracy and lack of patient educational resources to develop novel ways of utilising this technology. CGM is being used for diagnostics, monitoring changes in management, establishment of baseline characteristics, modifying behaviour, and ultimately to reduce hypoglycaemia when used retrospectively and combined with interpretative algorithms or clinical expertise. Use in neonatal medicine is becoming established, with good evidence for a reduction and early recognition in neonatal hypoglycaemia.

A lack of guidelines for the use of CGM in hypoglycaemia disorders has restricted progress but given rapid technological advances, it is predicted to play a larger role in all forms of childhood hypoglycaemia disorders. The challenge is to adapt CGM technology to clinical application with research designed to bring CGM innovations for patient benefit.

Author contributions

CW researched and wrote the first draft of the manuscript other than Section 5.2 which was written by LH. All authors contributed to the article and approved the submitted version.

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. Kristensen K, Quitmann J, Witt S. Health-related quality of life of children and adolescents with congenital hyperinsulinism – a scoping review. *Front Endocrinol (Lausanne)* (2021) 12:784932. doi: 10.3389/fendo.2021.784932

2. Pasquini TLS, Mesfin M, Schmitt J, Raskin J. Global registries in congenital hyperinsulinism. *Front Endocrinol (Lausanne)* (2022) 13:876903. doi: 10.3389/fendo.2022.876903

3. Eljamel S, Griffiths A, Evans J, Banerjee I, Hussain K, Thompson R. The burden of congenital hyperinsulinism in the united kingdom: A cost of illness study. *Orphanet J Rare Dis* (2018) 13. doi: 10.1186/s13023-018-0867-6

4. Wild D, von Maltzahn R, Brohan E, Christensen T, Clauson P, Gonder-Frederick L. A critical review of the literature on fear of hypoglycemia in diabetes: Implications for diabetes management and patient education. *Patient Educ Couns* (2007) 68:10–5. doi: 10.1016/J.PEC.2007.05.003

5. Nordfeldt S, Jonsson D. Short-term effects of severe hypoglycaemia in children and adolescents with type 1 diabetes. *A cost-of-illness study Acta Pædiatrica* (2001) 90:137–42. doi: 10.1111/J.1651-2227.2001.TB00274.X

6. Raskin J, Pasquini TLS, Bose S, Tallis D, Schmitt J. Congenital hyperinsulinism international: A community focused on improving the lives of people living with congenital hyperinsulinism. *Front Endocrinol (Lausanne)* (2022) 13:886552. doi: 10.3389/fendo.2022.886552

7. Shah R, Dai DWT, Alsweiler JM, Brown GTL, Chase JG, Gamble GD, et al. Association of neonatal hypoglycemia with academic performance in mid-childhood. *Jama* (2022) 327:1158. doi: 10.1001/jama.2022.0992

8. McKinlay CJD, 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) 373:1507–18. doi: 10.1056/NEJMoa1504909

9. McKinlay CJD, Alsweiler JM, Anstice NS, Burakevych N, Chakraborty A, Chase JG, et al. Association of neonatal glycemia with neurodevelopmental outcomes at 4.5 years. *JAMA Pediatr* (2017) 171:972–83. doi: 10.1001/jamapediatrics.2017.1579

10. Lucas A, Morley R, Cole TJ. Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. (1988), *British Medical Journal*, Vol 297 1304–8. doi: 10.1136/bmj.297.6659.1304

11. 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

12. 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

13. Worth C, Dunne M, Ghosh A, Harper S, Banerjee I. Continuous glucose monitoring for hypoglycaemia in children: Perspectives in 2020. *Pediatr Diabetes* (2020) 21:1–10. doi: 10.1111/pedi.13029

14. Šoupal J, Petruželkov´a L, Grunberger G, H´askov´a A, Flekač M, Matoulek M, et al. Glycemic outcomes in adults with T1D are impacted more by continuous glucose monitoring than by insulin delivery method: 3 years of follow-up from the COMISAIR study. *Diabetes Care* (2020) 43:37–43. doi: 10.2337/DC19-0888

15. Charleer S, De Block C, Van Huffel L, Broos B, Fieuws S, Nobels F, et al. Quality of life and glucose control after 1 year of nationwide reimbursement of intermittently scanned continuous glucose monitoring in adults living with type 1 diabetes (FUTURE): A prospective observational real-world cohort study. *Diabetes Care* (2020) 43:389–97. doi: 10.2337/DC19-1610

16. Wright EE, Kerr MSD, Reyes IJ, Nabutovsky Y, Miller E. Use of flash continuous glucose monitoring is associated with A1C reduction in people with type 2 diabetes treated with basal insulin or noninsulin therapy. *Diabetes Spectr* (2021) 34:184. doi: 10.2337/DS20-0069

17. Tauschmann M, Hermann JM, Freiberg C, Papsch M, Thon A, Heidtmann B, et al. Reduction in diabetic ketoacidosis and severe hypoglycemia in pediatric type 1 diabetes during the first year of continuous glucose monitoring: A multicenter analysis of 3,553 subjects from the DPV registry. *Diabetes Care* (2020) 43:e40–2. doi: 10.2337/DC19-1358

18. Wan W, Skandari MR, Minc A, Nathan AG, Winn A, Zarei P, et al. Cost-effectiveness of continuous glucose monitoring for adults with type 1 diabetes compared with self-monitoring of blood glucose: The DIAMOND randomized trial. *Diabetes Care* (2018) 41:1227–34. doi: 10.2337/DC17-1821

19. Roze S, Isitt J, Smith-Palmer J, Javanbakht M, Lynch P. Long-term cost-effectiveness of dexcom G6 real-time continuous glucose monitoring versus self-monitoring of blood glucose in patients with type 1 diabetes in the U.K. *Diabetes Care* (2020) 43:2411–7. doi: 10.2337/DC19-2213

20. Fonda SJ, Graham C, Munakata J, Powers JM, Price D, Vigersky RA. The cost-effectiveness of real-time continuous glucose monitoring (RT-CGM) in type 2 diabetes. *J Diabetes Sci Technol* (2016) 10:898–904. doi: 10.1177/1932296816628547

21. Beardsall K, Ogilvy-Stuart AL, Ahluwalia J, Thompson M, Dunger DB. The continuous glucose monitoring sensor in neonatal intensive care. *Arch Dis Child Fetal Neonatal Ed* (2005) 90:307–10. doi: 10.1136/adc.2004.051979

22. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart AL, Vanhole C, VanWeissenbruch M, Midgley P, et al. Validation of the continuous glucose monitoring sensor in preterm infants. *Arch Dis Child Fetal Neonatal Ed* (2013) 98:136–40. doi: 10.1136/archdischild-2012-301661

23. Win M, Beckett R, Thomson L, Thankamony A, Beardsall K. Continuous glucose monitoring in the management of neonates with persistent hypoglycaemia and congenital hyperinsulinism. *J Clin Endocrinol Metab* (2020) 107:1–18. doi: 10.1210/clinem/dgab601

24. 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

25. 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. doi: 10.1186/s13633-018-0057-2

26. Rayannavar A, Elci OU, Mitteer L, De León DD. Continuous glucose monitoring systems: Are they useful for evaluating glycemic control in children with hyperinsulinism? *Horm Res Paediatr* (2020) 92:319–27. doi: 10.1159/000506230

27. Worth C, Dunne MJ, Salomon-Estebanez M, Harper S, Nutter PW, Dastamani A, et al. The hypoglycaemia error grid: a UK-wide consensus on CGM accuracy assessment in hyperinsulinism. *Front Endocrinol (Lausanne)* (2022). doi: 10.3389/fendo.2022.1016072

28. Kasapkara CS, Cinasal Demir G, Hasanoğlu 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

29. Herbert M, Pendyal S, Rairikar M, Halaby C, Benjamin RW, Kishnani PS. Role of continuous glucose monitoring in the management of glycogen storage disorders. *J Inherit Metab Dis* (2018) 41:917–27. doi: 10.1007/s10545-018-0200-5

30. Rossi A, Venema A, Haarsma P, Feldbrugge L, Burghard R, Rodriguez-Buritica D, et al. A prospective study on continuous glucose monitoring in glycogen storage disease type ia: Toward glycemic targets. *J Clin Endocrinol Metab* (2022) 107:e3612–23. doi: 10.1210/clinem/dgac411

31. Fernández Martínez M del M, Llorente JLG, de Cabo JM, López MAV, Porcel M del CO, Rubio JDD, et al. Monitoring the frequency and duration of hypoglycemia in preterm infants and identifying associated factors. *Fetal Pediatr Pathol* (2021) 40:131–41. doi: 10.1080/15513815.2019.1692111

32. 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 Heal* (2021) 5:265–73. doi: 10.1016/S2352-4642(20)30367-9

33. Denkboy Ongen Y, Eren E, Saglam H. Maltodextrin may be a promising treatment modality after near-total pancreatectomy in infants younger than six months with persistent hyperinsulinism: A case report. *J Clin Res Pediatr Endocrinol* (2012) 0:0–0. doi: 10.4274/jcrpe.galenos.2021.2021.0121

34. Kiuchi K, Sato T, Nakano S, Ishii T, Hasegawa T. Continuous glucose monitoring in an infant with panhypopituitarism having hypoglycemia on growth hormone therapy. *J Pediatr Endocrinol Metab* (2022) 2975:1–4. doi: 10.1515/jpem-2022-0224

35. Worth C, Nutter PW, Salomon-Estebanez M, Auckburally S, Dunne MJ, Banerjee I, et al. The behaviour change behind a successful pilot of hypoglycaemia reduction with HYPO-CHEAT. *Under Rev Digit Heal* (2022).

36. White FJ, Jones SA. The use of continuous glucose monitoring in the practical management of glycogen storage disorders. *J Inherit Metab Dis* (2011) 34:631–42. doi: 10.1007/s10545-011-9335-3

37. Du CQ, Wei H, Zhang C, Zhang M, Hu MH, Hou L, et al. [The application of continuous glucose monitoring in the management of hepatic glycogen storage disease]. *Chin J Pediatr* (2021) 59:452–8. doi: 10.3760/cma.j.cn112140-20210321-00235

38. 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 Genet Metab* (2019) 126:355–61. doi: 10.1016/j.ymgme.2019.02.008

39. Esper BS, Carson M, Galvin-Parton P, Lane AH, Tafuri KS. Continuous glucose monitoring in the management of hypoglycemia in TANGO2. *J Endocr Soc* (2021) 5:A699–9. doi: 10.1210/jendso/bvab048.1423

40. Ng SM, Dearman S, Fisher M, Mushtaq T. Case for funding of continuous glucose monitoring systems for patients with recurrent hypoglycaemia. *Arch Dis Child* (2022), 1–2. doi: 10.1136/archdischild-2022-323872

41. Peeks F, Boonstra WF, De BL, Carøe C. Research priorities for liver glycogen storage disease : An international priority setting partnership with the. *J Inherit Metab Dis* (2020) 43:279–89. doi: 10.1002/jimd.12178

42. 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) 44:1136–50. doi: 10.1002/jimd.12383

43. Auckburally S, Worth C, Salomon-Estebanez M, Nutter PW, Harper S, Nicholson J, et al. Families' experiences of continuous glucose monitoring in the management of congenital hyperinsulinism: A thematic analysis. *Front Endocrinol (Lausanne)* (2022). doi: 10.3389/fendo.2022.894559

44. Ahmad S, Worth C, Auckburally S, Salomon-Estebanez M, O'Shea E, Worthington S, et al. Continuous glucose monitoring for hypoglycaemia: the unheard patient voice IN 60th annual meeting of the European society for paediatric endocrinology (ESPE)(2022). Available at: https://abstracts.eurospe.org/hrp/0095/hrp0095p1-295 (Accessed November 3, 2022).

45. UnitedNations. The sustainable development goals report 2022. *United Nations Publ issued by Dep Econ Soc Aff* (2022) 64.

46. Bila R, Varo R, Madrid L, Sitoe A, Bassat Q. Continuous glucose monitoring in resource-constrained settings for hypoglycaemia detection: Looking at the problem from the other side of the coin. *Biosensors* (2018) 8:2–7. doi: 10.3390/bios8020043

47. Martínez C, Romero C, Villalobos NS. *What are pharma companies doing to expand access to insulin – and how can efforts be scaled up?* (2022). Available at: https://accesstomedicinefoundation.org/medialibrary/633ed0a126443_atmf-diabetes_access-to-insulin.pdf.

48. Newborn mortality, in: *World heal organ* (2022). Available at: https://www.who.int/news-room/fact-sheets/detail/levels-and-trends-in-child-mortality-report-2021 (Accessed November 16, 2022).

49. Anderson S, Shakya KN, Shrestha LN, De AM. Hypoglycaemia: A common problem among uncomplicated newborn infants in Nepal. *J Trop Pediatr* (1993) 39:273–7. doi: 10.1093/tropej/39.5.273

50. Maynard KR, Causey L, Kawaza K, Dube Q, Lufesi N, Oden ZM, et al. New technologies for essential newborn care in under-resourced areas: What is needed and how to deliver it. *Paediatr Int Child Health* (2015) 35:192–205. doi: 10.1179/2046905515Y.0000000034

51. Burns CM, Rutherford MA, Boardman JP, Cowan FM. Patterns of cerebral injury and neurodevelopmental outcomes after symptomatic neonatal hypoglycemia. *Pediatrics* (2008) 122:65–74. doi: 10.1542/peds.2007-2822

52. Kariuki SM, Thomas PT, Newton CR. Epilepsy stigma in children in low-income and middle-income countries. *Lancet Child Adolesc Heal* (2021) 5:314–6. doi: 10.1016/S2352-4642(21)00090-0

53. Dua T, De Boer HM, Prilipko LL, Saxena S. Epilepsy care in the world: Results of an ILAE/IBE/WHO global campaign against epilepsy survey. *Epilepsia* (2006) 47:1225–31. doi: 10.1111/j.1528-1167.2006.00595.x

54. Smythe T, Adelson JD, Polack S. Systematic review of interventions for reducing stigma experienced by children with disabilities and their families in low- and middle-income countries: state of the evidence. *Trop Med Int Heal* (2020) 25:508–24. doi: 10.1111/tmi.13388

55. Deerberg-Wittram J. *Diabeter value-based healthcare delivery in diabetes diabeter: Value-based health care delivery in diabetes 1*. (2016). NA - Medtronic online publication

56. Venema A, Peeks F, Rossi A, Jager EA, Derks TGJ. Towards values-based healthcare for inherited metabolic disorders: An overview of current practices for persons with liver glycogen storage disease and fatty acid oxidation disorders. *J Inherit Metab Dis* (2022) 45:1018–27. doi: 10.1002/jimd.12555

57. Schopman S, Kalchthaler K, Malthern R, Mehta K, Butler P. Ruggedising biomedical devices for field-testing in resource-constrained environments: Context, issues and solutions. *J Humanit Eng* (2013) 2:17–26. doi: 10.36479/jhe.v2i1.17

58. Howitt P, Darzi A, Yang GZ, Ashrafian H, Atun R, Barlow J, et al. Technologies for global health. *Lancet* (2012) 380:507–35. doi: 10.1016/S0140-6736(12)61127-1

59. Free MJ. Achieving appropriate design and widespread use of health care technologies in the developing world. overcoming obstacles that impede the adaptation and diffusion of priority technologies for primary health care. *Int J Gynecol Obstet* (2004) 85:3–13. doi: 10.1016/j.ijgo.2004.01.009

60. Kasali BA, Gururaj A, Batra M. Newborn care technology investments for LMIC settings: a CPAP approach. *BMJ Innov* (2021) 7:519–22. doi: 10.1136/bmjinnov-2020-000598

61. Ahmed I, Jiang N, Shao X, Elsherif M, Alam F, Salih A, et al. Recent advances in optical sensors for continuous glucose monitoring. *Sensors Diagnostics* (2022). doi: 10.1039/d1sd00030f

62. Zafar H, Channa A, Jeoti V, Stojanović GM. Comprehensive review on wearable sweat-glucose sensors for continuous glucose monitoring. *Sensors* (2022) 22:1–35. doi: 10.3390/s22020638

63. Kumar Das S, Nayak KK, Krishnaswamy PR, Kumar V, Bhat N. Review–electrochemistry and other emerging technologies for continuous glucose monitoring devices. *ECS Sensors Plus* (2022) 1:031601. doi: 10.1149/2754-2726/ac7abb

64. Oviedo S, Vehí J, Calm R, Armengol J. A review of personalized blood glucose prediction strategies for T1DM patients. *Int J numer method BioMed Eng* (2017) 33. doi: 10.1002/cnm.2833

65. Leelarathna L, English SW, Thabit H, Caldwell K, Allen JM, Kumareswaran K, et al. Feasibility of fully automated closed-loop glucose control using continuous subcutaneous glucose measurements in critical illness: A randomized controlled trial. *Crit Care* (2013) 17. doi: 10.1186/cc12838

66. Beardsall K, Thomson L, Elleri D, Dunger DB, Hovorka R. Feasibility of automated insulin delivery guided by continuous glucose monitoring in preterm infants. *Arch Dis Child Fetal Neonatal Ed* (2020) 105:F279–84. doi: 10.1136/archdischild-2019-316871

67. Bertachi A, Viñals C, Biagi L, Contreras I, Vehí J, Conget I, et al. Prediction of nocturnal hypoglycemia in adults with type 1 diabetes under multiple daily injections using continuous glucose monitoring and physical activity monitor. *Sensors 2020 Vol 20 Page 1705* (2020) 20:1705. doi: 10.3390/S20061705

68. Amar Y, Shilo S, Oron T, Amar E, Phillip M, Segal E. Clinically accurate prediction of glucose levels in patients with type 1 diabetes(2020). Available at: https://home.liebertpub.com/dia.

69. Elhadd T, Mall R, Bashir M, Palotti J, Fernandez-Luque L, Farooq F, et al. Artificial intelligence (AI) based machine learning models predict glucose variability and hypoglycaemia risk in patients with type 2 diabetes on a multiple drug regimen who fast during ramadan (The PROFAST – IT Ramadan study). *Diabetes Res Clin Pract* (2020) 169:108388. doi: 10.1016/J.DIABRES.2020.108388

70. Dave D, DeSalvo DJ, Haridas B, McKay S, Shenoy A, Koh CJ, et al. Feature-based machine learning model for real-time hypoglycemia prediction. *J Diabetes Sci Technol* (2020) 15(4), 842–855. doi: 10.1177/1932296820922622

71. Mujahid O, Contreras I, Vehi J. Machine learning techniques for hypoglycemia prediction: Trends and challenges. *Sensors (Switzerland)* (2021) 21:1–21. doi: 10.3390/s21020546

72. Kodama S, Fujihara K, Shiozaki H, Horikawa C, Yamada MH, Sato T, et al. Ability of current machine learning algorithms to predict and detect hypoglycemia in patients with diabetes mellitus: meta-analysis. *JMIR Diabetes* (2021) 6. doi: 10.2196/22458

73. Tyler NS, Mosquera-Lopez CM, Wilson LM, Dodier RH, Branigan DL, Gabo VB, et al. An artificial intelligence decision support system for the management of type 1 diabetes. *Nat Metab* (2020) 2:612–9. doi: 10.1038/s42255-020-0212-y

74. Liu C, Avari P, Leal Y, Wos M, Sivasithamparam K, Georgiou P, et al. A modular safety system for an insulin dose recommender: A feasibility study. *J Diabetes Sci Technol* (2020) 14:87–96. doi: 10.1177/1932296819851135

75. Tyler NS, Jacobs PG. Artificial intelligence in decision support systems for type 1 diabetes. *Sensors (Switzerland)* (2020) 20:1–26. doi: 10.3390/s20113214

76. Nano J, Carinci F, Okunade O, Whittaker S, Walbaum M, Barnard-Kelly K, et al. A standard set of person-centred outcomes for diabetes mellitus: results of an international and unified approach. *Diabetes Med* (2020) 37:2009–18. doi: 10.1111/dme.14286

77. Agiostratidou G, Anhalt H, Ball D, Blonde L, Gourgari E, Harriman KN, et al. Standardizing clinically meaningful outcome measures beyond HbA1c for type 1 diabetes: A consensus report of the American association of clinical endocrinologists, the American association of diabetes educators, the American diabetes association, the endo. *Diabetes Care* (2017) 40:1622–30. doi: 10.2337/dc17-1624

78. Jain SH, Powers BW, Hawkins JB, Brownstein JS. The digital phenotype. *Nat Biotechnol* (2015) 33:462–3. doi: 10.1038/nbt.3223

79. Flores M, Glusman G, Brogaard K, Price ND, Hood L. P4 medicine: How systems medicine will transform the healthcare sector and society. *Per Med* (2013) 10:565–76. doi: 10.2217/pme.13.57

80. Worth C, Harper S, Salomon-Estebanez M, O'Shea E, Nutter PW, Dunne MJ. Indraneel Banerjee,MBBS m. clustering of hypoglycemia events in patients with hyperinsulinism: Extension of the digital phenotype through retrospective data analysis. *J Med Internet Res* (2021) 23:1–14. doi: 10.2196/26957

81. Worth C, Nutter PW, Dunne MJ, Salomon-Estebanez M, Banerjee I, Harper S. HYPO-CHEAT's aggregated weekly visualisations of risk reduce real world hypoglycaemia. *Digit Heal* (2022) 8:1–22. doi: 10.1177/20552076221129712

82. Worth C, Tropeano Y, Gokul PR, Cosgrove KE, Salomon-Estebanez M, Senniappan S, et al. Insight into hypoglycemia frequency in congenital hyperinsulinism: evaluation of a large UK CGM dataset. *BMJ Open Diabetes Res Care* (2022) 10:e002849. doi: 10.1136/BMJDRC-2022-002849

83. Park J, Hawcutt D, Shantsila H, Lip G, Blair J. Glucose regulation in children with primary adrenal insufficiency: preliminary data(2021). Available at: https://abstracts.eurospe.org/hrp/0094/hrp0094p1-10 (Accessed November 7, 2022).

84. Woldaregay AZ, Årsand E, Walderhaug S, Albers D, Mamykina L, Botsis T, et al. Data-driven modeling and prediction of blood glucose dynamics: Machine learning applications in type 1 diabetes. *Artif Intell Med* (2019) 98:109–34. doi: 10.1016/j.artmed.2019.07.007

85. Ehrhardt N, Al Zaghal E. Behavior modification in prediabetes and diabetes: Potential use of real-time continuous glucose monitoring. *J Diabetes Sci Technol* (2019) 13:271–5. doi: 10.1177/1932296818790994

86. Ehrhardt N, Al Zaghal E. Continuous glucose monitoring as a behavior modification tool. *Clin Diabetes* (2020) 38:126–31. doi: 10.2337/cd19-0037

87. Schembre SM, Liao Y, Jospe MR. Continuous glucose monitors as wearable lifestyle behavior change tools in obesity and diabetes. *Obes Diabetes* (2020), 591–603. doi: 10.1007/978-3-030-53370-0_43

88. Lee SK, Shin DH, Kim YH, Lee KS. Effect of diabetes education through pattern management on self-care and self-efficacy in patients with type 2 diabetes. *Int J Environ Res Public Health* (2019) 16. doi: 10.3390/ijerph16183323

89. 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) 104:4356–64. doi: 10.1210/jc.2018-02763

90. Dubose SN, Kanapka LG, Bradfield B, Sooy M, Beck RW, Steck AK. Continuous glucose monitoring profiles in healthy, nondiabetic young children. *J Endocr Soc* (2022) 6:1–7. doi: 10.1210/jendso/bvac060

91. Rossi A, Rutten MGS, van Dijk TH, Bakker BM, Reijngoud DJ, Oosterveer MH, et al. Dynamic methods for childhood hypoglycemia phenotyping: A narrative review. *Front Endocrinol (Lausanne)* (2022) 13:858832. doi: 10.3389/fendo.2022.858832

92. Logel SN, Connor EL, Hsu DA, Fenske RJ, Paloian NJ, De Vivo DC. Exploring diazoxide and continuous glucose monitoring as treatment for Glut1 deficiency syndrome. *Ann Clin Transl Neurol* (2021) 8:2205–9. doi: 10.1002/ACN3.51462

93. Sadhwani A, Asaro LA, Goldberg CS, Ware J, Butcher J, Gaies M, et al. Impact of tight glycemic control and hypoglycemia after pediatric cardiac surgery on neurodevelopmental outcomes at three years of age: Findings from a randomized clinical trial. *BMC Pediatr* (2022) 22:1–10. doi: 10.1186/s12887-022-03556-z

94. Banerjee I, De Leon DD, Kendall DM, Birch S, Bøge E, Ivkovic J, et al. Dasiglucagon treatment over 21 days in infants with congenital hyperinsulinism results in glycaemic stability and reduces requirement for intravenous glucose IN 60th annual meeting of the European society for paediatric endocrinology (ESPE). *Horm Res Paediatr* (2022) 95(2). doi: 10.1159/000525606

95. Demirbilek H, Melikyan M, Galcheva S, Dastamani A, Thornton P, De Leon D, et al. Results from a global, multi-center, phase 2b study (RIZE) in congenital hyperinsulinism: Characterization of a high unmet treatment need and glycemic response to RZ358 IN 60th annual meeting of the European society for paediatric endocrinology (ESPE). *Horm Res Paediatr* (2022) 95(2). doi: 10.1159/000525606

96. NIH. *ClinicalTrials: A study of adeno-associated virus serotype 8-mediated gene transfer of glucose-6-Phosphatase in patients with glycogen storage disease type ia (GSDIa)* (2021). Available at: https://clinicaltrials.gov/ct2/show/NCT05139316 (Accessed December 2, 2022).

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K_{ATP} channel mutations in congenital hyperinsulinism: Progress and challenges towards mechanism-based therapies

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Congenital hyperinsulinism (CHI) is the most common cause of persistent hypoglycemia in infancy/childhood and is a serious condition associated with severe recurrent attacks of hypoglycemia due to dysregulated insulin secretion. Timely diagnosis and effective treatment are crucial to prevent severe hypoglycemia that may lead to life-long neurological complications. In pancreatic β-cells, adenosine triphosphate (ATP)-sensitive K^+ (K_{ATP}) channels are a central regulator of insulin secretion vital for glucose homeostasis. Genetic defects that lead to loss of expression or function of K_{ATP} channels are the most common cause of HI (K_{ATP}-HI). Much progress has been made in our understanding of the molecular genetics and pathophysiology of K_{ATP}-HI in the past decades; however, treatment remains challenging, in particular for patients with diffuse disease who do not respond to the K_{ATP} channel activator diazoxide. In this review, we discuss current approaches and limitations on the diagnosis and treatment of K_{ATP}-HI, and offer perspectives on alternative therapeutic strategies.

KEYWORDS

ATP-sensitive potassium channel, insulin secretion, sulphonylurea receptor 1 (SUR1), kir6.2, hypoglycemia

Introduction

Congenital hyperinsulinism (CHI) is a group of clinically, genetically, and morphologically heterogeneous disorders characterized by recurrent episodes of hyperinsulinemia and hypoglycemia due to dysregulated insulin secretion from pancreatic β-cells (1, 2). This condition can lead to neonatal seizures, developmental delay, and irreversible brain damage if not promptly diagnosed and treated (3). The age of clinical presentation in general correlates with the severity of the disease (4). Severe cases show symptoms of hypoglycemia early in the neonatal life, while milder forms are usually diagnosed later in infancy or childhood with recurrent attacks of hypoglycemia, which manifest following prolonged fasting or other health stress. CHI was first named as

"idiopathic hypoglycemia of infancy" (5), which is no longer used after many genetic causes of the disease have been identified (6, 7). It was also once referred to as "nesidioblastosis" based on an early suggestion that the increased insulin secretion is secondary to budding of pancreatic islets observed in histological samples from patients with CHI (8). The use of this name to describe CHI was discontinued after nesidioblastosis was revealed to be a normal fetal and neonatal phenomenon (9, 10). Another discontinued historical term is persistent hyperinsulinemic hypoglycemia of infancy or PHHI, as it is now understood that the disease can be neonatal, infantile or childhood and can persist to adulthood (11). In addition to prototypical CHI, hyperinsulinism can be a pathology of a syndromic disease, including Beckwith-Wiedemann syndrome, Perlman syndrome, Kabuki syndrome, Turner syndrome, Sotos syndrome, and others (7, 12).

The estimated incidence of CHI is 1:28,000–1:50,000 in Western populations but as high as 1:2,500 in populations with higher rates of consanguinity (3, 13, 14). Variants in at least ten genes have now been linked to congenital hyperinsulinism, including genes that encode the K_{ATP} channel subunits (*ABCC8* and *KCNJ11*), glucokinase (*GCK*), glutamate dehydrogenase (*GLUD1*), the mitochondrial enzyme 3-hydroxyacyl-CoA dehydrogenase (*HADH*), proton-linked monocarboxylate transporter (*SLC16A1*), mitochondrial uncoupling protein 2 (*UCP2*), hepatocyte nuclear factor 1 alpha (*HNF1A*) and 4 alpha (*HNF4A*), and hexokinase 1 (*HK-1*) (15). Defects in these proteins result in dysregulation of insulin secretion and impaired glucose homeostasis. Of the CHI-associated gene mutations, those in *ABCC8* or *KCNJ11* that lead to loss-of-function of K_{ATP} channels are the most common (16). The majority of K_{ATP} gene mutations identified to date are in *ABCC8*, which is much larger than *KCNJ11* (16). This review summarizes current approaches and limitations on the diagnosis and treatment of K_{ATP}-HI, and offers perspectives on alternative therapeutic strategies. Readers interested in K_{ATP} channel structure-function and pharmacology are referred to several recent reviews (17–19).

K_{ATP}-HI: Molecular diagnosis

K_{ATP} channels have a central role in regulating insulin secretion from pancreatic β-cells in the islets of Langerhans (20, 21). The channel is composed of four pore-forming subunits Kir6.2, encoded by *KCNJ11*, and four regulatory subunits called sulfonylurea receptor 1 (SUR1), encoded by *ABCC8* (22, 23) (Figures 1A, B). SUR1 is so named because it binds sulfonylurea drugs, which inhibit K_{ATP} channel activity and are commonly used to treat type 2 diabetes (24). Pancreatic K_{ATP} channels are gated physiologically by intracellular ATP and ADP; ATP acts on Kir6.2 to close the channel, while MgADP acts on SUR1 to open the channel (17). This enables K_{ATP} channels to serve as metabolic sensors, coupling serum glucose to insulin secretion. At basal glucose levels, the ATP/ADP ratios are relatively low to allow K^+ conductance through K_{ATP} channels, which sets the plasma membrane in a hyperpolarized state to prevent insulin secretion. When blood glucose levels rise, glucose metabolism increases the ATP/ADP ratio, which favors closure of K_{ATP} channels, resulting in cell membrane depolarization, activation of voltage-gated Ca^{2+} channels, and exocytosis of insulin granules (25) (Figure 1C). The ability of K_{ATP} channels to control β-cell membrane excitability in response to blood glucose levels is essential for glucose homeostasis. In CHI, faulty K_{ATP} channel genes that reduce or abolish functional channels in the β-cell membranes uncouple blood glucose from insulin secretion, leading to inappropriate insulin secretion despite life-threatening hypoglycemia (21, 26, 27).

Molecular diagnosis of K_{ATP}-HI begins with genetic testing. Genomic DNA mutation screening of probands is done using genome isolated from peripheral blood or saliva (7, 28). However, interpreting the effect of novel *ABCC8* or *KCNJ11* variants can be challenging as they can be dominant or recessive functional mutations, or benign polymorphisms (16, 29). Recent advances combining genetic, clinical, and *in vitro* biochemical studies to determine which of the genetic variations affect transcription/splicing, translation, and function have significantly improved the diagnosis of K_{ATP}-HI (30, 31).

Focal versus diffuse K_{ATP}-HI

Histologically, there exist two forms of K_{ATP}-HI: focal and diffuse (15, 32). In focal disease, the defect is limited to a focal lesion in the pancreas due to a heterozygous, paternally inherited *ABCC8* or *KCNJ11* mutation coupled with loss of the normal maternal allele in a subset of pancreatic β-cells during embryonic development (28). In diffuse K_{ATP}-HI the entire pancreas is affected. Patients carrying homozygous, heterozygous mutations, or rare compound heterozygous mutations have all been reported (29). The two forms of K_{ATP}-HI are not easily distinguishable by clinical presentations (15). Genetic information from the proband and parents can point to possible focal disease. Indeed, in patients whose genetic testing identifies one paternally inherited recessive mutation in *ABCC8* or *KCNJ11* and no maternal mutation, there is > 95% likelihood of a focal disease (29). This can be directly confirmed by PET imaging using ^{18}F-fluoro-L-DOPA. Localization of focal lesions by ^{18}F-fluoro-L-DOPA PET imaging along with CT-angiography allows for surgical removal of focal lesions in most cases for a complete cure (33). By contrast, the diffuse form, if not responsive to pharmacological treatment or glucose infusion, may require near total pancreatectomy to manage hypoglycemia, leading to future complications (3, 34).

Dominant versus recessive K_{ATP}-HI

Determining whether the mutation is dominant or recessive is of great clinical importance, especially for guiding decisions on whether to conduct ^{18}F-fluoro-L-DOPA PET scan to test for focal disease. Moreover, the information is important for genetic counseling concerning recurrence risk as well as for identifying other family members at risk for hypoglycemia (35, 36). In focal CHI, a paternally inherited faulty gene only manifests in a focal region of cells where loss of heterozygosity of the maternal allele

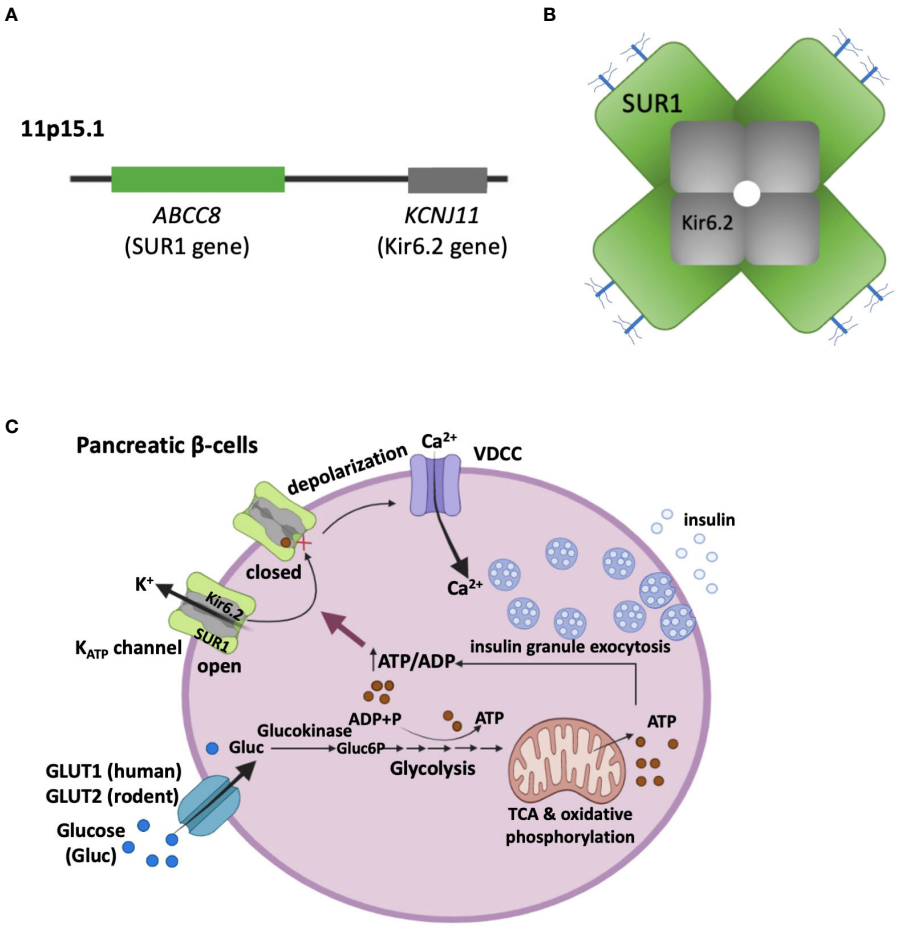

FIGURE 1
K_{ATP} channel composition and its role in coupling glucose metabolism to insulin secretion. **(A)** *ABCC8* and *KCNJ11* encoding the two pancreatic K_{ATP} channel subunits, SUR1 and Kir6.2 respectively, are located on the short arm of chromosome 11 (11p15.1). **(B)** Schematic representation of the K_{ATP} channel complex viewed in cross section. The K^+-conducting pore is formed by four Kir6.2 subunits, which are surrounded by four SUR1 regulatory subunits. The branched blue sticks represent the two N-linked glycosylation sites in each SUR1. **(C)** β-cell K_{ATP} channels couple glucose metabolism to insulin secretion by regulating plasma membrane potential in response to varying blood glucose levels. With high serum blood glucose levels, glucose enters β-cells through glucose transporters (GLUT1 in human, and GLUT2 in rodents). Inside the cell, glucose is catabolized in the cytosol (Glycolysis) and the mitochondria (Tricarboxylic acid cycle, TCA), leading to an elevation of the ATP/ADP ratio. This results in closure of K_{ATP} channels, plasma membrane depolarization, opening of voltage-dependent Ca^{2+} channels (VDCC). The ensuing Ca^{2+} influx then triggers exocytosis of insulin secretory granules.

occurs, but not in the rest of the pancreas. Thus K_{ATP} mutations identified in focal CHI are recessive. Since both inheritance of the paternal mutant allele and loss of the maternal allele are required for disease presentation, the recurrence among the siblings is rare; to our knowledge, it has only been reported once in two siblings (37). However, in consanguineous parents, mothers should be screened for the presence of the paternal mutation responsible for the focal form of CHI to avoid the possibility of diffuse CHI in future pregnancies due to inheritance of the mutation from both parents.

Unlike focal K_{ATP}-HI, diffuse K_{ATP}-HI may be dominant or recessive, which often correlates with whether the underlying mutation causes K_{ATP} channel gating or trafficking defects (see more discussion in "Mechanisms of K_{ATP}-HI mutations"). Recessive mutations, whether homozygous or compound heterozygous, are usually found in patients with severe disease and not responsive to diazoxide treatment (38). Dominant mutations have been identified in patients with mild, diazoxide-responsive disease as well as severe, diazoxide-unresponsive disease (39–43). For genetic counseling, homozygous recessive mutations are expected to have a recurrence rate of ~25%, while dominant heterozygous mutations have a recurrence rate up to 50%. Differentiating between dominant and recessive mutations can in some cases be challenging. For example, penetrance of a mutation may not be the same in all carriers (44). Differential expression of a dominant *ABCC8* mutation has been observed in lymphocytes from two different carriers and proposed to account for the difference in their clinical presentation of CHI (45). Moreover, the pedigrees of CHI patients are often too small to clarify the inheritance pattern of novel mutations. Combining clinical, genetic, and functional studies using *in vitro* recombination systems can help resolve ambiguous cases.

Mechanisms of K_{ATP}-HI mutations

ABCC8 contains 42 exons encoding 1581 amino acids (or a common alternative isoform with 1582 residues), whereas *KCNJ11* contains a single exon encoding 390 amino acids. Genetic variants in *ABCC8* and *KCNJ11* are found in both non-coding and coding regions. We have divided them into three broad classes: those causing impairment of transcription and translation, those disrupting folding, assembly, and trafficking, and those disrupting gating of K_{ATP} channels (Figure 2).

In non-coding regions, genetic variations can in principle affect gene expression, or splicing in the case of *ABCC8* to reduce transcript copies, and thereby K_{ATP} expression and function. Few studies have examined regulation of *ABCC8* and *KCNJ11* genes in pancreatic β-cells by cis-elements in the non-coding regions (46, 47). Definitive demonstration that a variation in these regions reduce transcript number would be very difficult. Several studies have presented *in vitro* evidence that genetic variations could disrupt splicing of SUR1 transcripts, especially variants located near the *ABCC8* intron-exon boundaries, using a combination of bioinformatics and expression of mini-genes containing the variants or digital droplet PCR of patient lymphocytes (31, 48–51).

Mutations in the coding regions can introduce premature stop codons or frameshift, resulting in truncated, nonfunctional proteins, but can also be silent, i.e. without changing the encoded amino acid. Silent mutations could potentially affect protein translation and folding by altering mRNA structure or codon usage (52); however, this possibility has not been tested. Most commonly, mutations in the coding regions alter primary sequence of SUR1 or Kir6.2. These include missense mutations and indel mutations. Alterations in the primary sequence of channel proteins can reduce or abolish channel function by disrupting channel folding, assembly, trafficking and/or gating response to blood glucose levels (26, 27, 53).

Understanding how novel K_{ATP} channel missense/indel mutations affect channel expression and function greatly facilitates molecular diagnosis and therapeutic management of CHI. Currently, *in silico* methods are unable to accurately predict the functional impact of a mutation, and native β-cells from patients are mostly unavailable. Endocrinologists around the world collaborate with several academic laboratories including ours to characterize effects of these mutations in recombinant expression systems using biochemical and electrophysiological assays (54). For these studies, recombinant mutant channels are transiently expressed in a mammalian cell line that does not express endogenous K_{ATP} channels, such as COSm6 or HEK293 cells. To assess the impact of a mutation on channel properties, mutant channels are first expressed and evaluated as a homogeneous population mimicking homozygous state. For heterozygous mutations, follow-up studies where the mutant is co-expressed with the wild-type at 1:1 ratio to simulate the heterozygous state may be performed to determine whether a mutation has a dominant effect over the wild-type allele on channel expression and function (30).

A rapid and informative method to determine whether a mutation is pathogenic is the Rb^+ efflux assay (54, 55). In this assay, Rb^+, which passes through K_{ATP} channels, is a tracer ion that acts as a surrogate K^+ and can be detected using a radioactive form of Rb^+, $^{86}Rb^+$ (54), or by atomic absorption spectroscopy (56), to monitor K_{ATP} channel activity. In β-cells, K_{ATP} channels open in response to glucose deprivation, which lowers the intracellular ATP/ADP ratio. In COSm6 cells, which are not glucose-responsive, reduction of ATP/ADP ratios can be triggered by incubating cells with metabolic inhibitors including the glycolysis

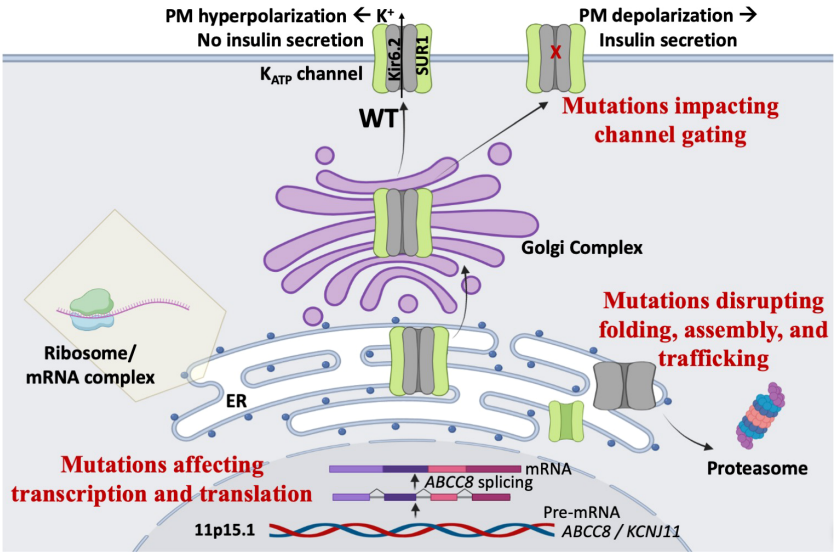

FIGURE 2
Mechanisms of K_{ATP}-HI mutations. Mutations in the K_{ATP} channel genes can lead to loss of channel function, persistent plasma membrane (PM) depolarization, and inappropriate insulin secretion *via* multiple mechanisms. First, mutations may impair gene transcription or protein translation. Second, mutations may disrupt K_{ATP} channel protein folding, assembly, and trafficking, thereby compromising surface expression of the channel. Third, mutations may cause gating defects that prevent channel opening when blood glucose levels decline.

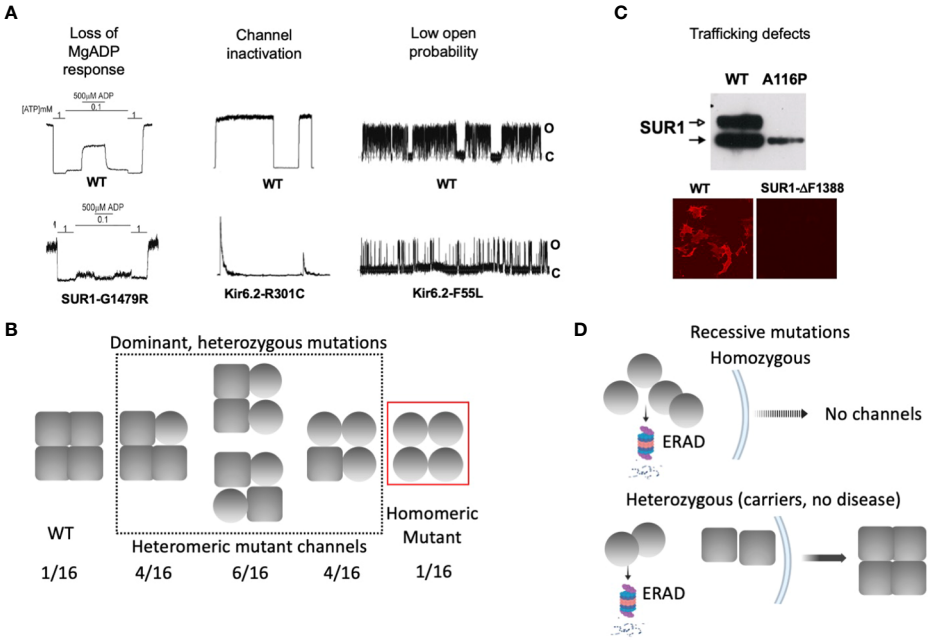

FIGURE 3
Biochemical and functional characteristics of K_{ATP} channel mutations associated with dominant versus recessive diffuse CHI. **(A)** Gating defects that have been observed in CHI-associated mutations, including loss of MgADP response, spontaneous current decay. i.e. channel inactivation, and reduced channel open probability. For each defect, representative inside-out patch-clamp recordings of WT and homomeric mutant channels are shown. For monitoring MgADP response, channels were exposed to 1 mM ATP, 0.1 mM ATP, or 0.1 mM ATP plus 0.5 mM MgADP as indicated. In all recordings, channel openings (O) are shown as upward deflections, and channel closures **(C)** as flat baselines. **(B)** Schematic showing that gating mutations tend to be associated with dominant disease, where heterozygous patients are expected to express a mixed population of channels containing 0-4 mutant subunits. In these cases, the mutation causes gating defects but not defects in channel folding, assembly, and trafficking. **(C)** *Top*: A western blot showing both core-glycosylated and complex-glycosylated wild-type SUR1 band when co-expressed with Kir6.2, indicating channel assembly and ability to traffic to the cell surface. In contrast, a trafficking mutation SUR1-A116P, fails to generate the mature complex-glycosylated band. *Bottom*: Surface immunofluorescence staining showing lack of expression of a trafficking mutant SUR1-ΔF1388, in contrast to WT. **(D)** Schematic showing trafficking mutations are usually seen in recessive disease. In patients carrying homozygous mutations, mutant protein is targeted for ER-associated proteasomal degradation (ERAD) and is unable to form channels and traffic to the plasma membrane. In heterozygous individuals, mutant protein is degraded and unable to assemble with WT subunit, leaving WT protein to assemble into functional channels that traffic to the plasma membrane, escaping the disease.

inhibitor 2-deoxyglucose and the oxidative phosphorylation inhibitor oligomycin, which reduce ATP production. In COSm6 cells transiently expressing K_{ATP} channels and preloaded with Rb^+, opening of K_{ATP} channels leads to increased Rb^+ efflux. Reduced efflux observed in cells expressing channels harboring a mutation compared to cells expressing wild-type channels would indicate that the mutation causes loss of channel function, therefore has a pathogenic role in CHI. In addition to its utility in evaluating the pathogenic role of a mutation, the Rb^+ efflux assay is also useful for assessing whether a mutant form of the channel functionally responds to the K_{ATP} channel opener diazoxide, a frontline treatment for CHI (57). Restoration of Rb^+ efflux by diazoxide would indicate a clinical response of patients with the mutation to diazoxide treatment. Indeed, there is in general good agreement between response in Rb^+ efflux assays and clinical response to diazoxide based on published work (29, 40, 42, 58). However, phenotypical variations caused by the same mutation could result in patient response that deviates from prediction based on Rb^+ efflux assays using recombinant mutant channels expressed in cultured cells.

Mutations which reduce K_{ATP} channel activity in Rb^+ efflux assays can disrupt the ability of the channel to open at low glucose concentrations (gating defects) and/or reduce the number of channels present in the plasma membrane (trafficking defects). The precise mechanisms can be further determined using biochemical and electrophysiological assays, which will aid in the decision on disease treatment plans.

Mutations disrupting channel gating

Accurate response of K_{ATP} channels to changes in intracellular ATP and MgADP concentrations is essential for glucose-insulin secretion coupling (25). In addition, channel activity relies on interactions with membrane phospholipids, in particular $PI4,5P_2$ (PIP_2) (59, 60). Patch-clamp recordings of K_{ATP} channels using the inside-out configuration allows for precise control of the solution on the intracellular face of channels contained in a membrane patch to evaluate channel response to the above physiological ligands. The most common gating defect seen in CHI-associated mutations is impaired response to MgADP/MgATP (61, 62) (Figure 3A), which stimulates K_{ATP} channels by binding to the SUR1 nucleotide binding domains (NBDs). Accordingly, these mutations are almost exclusively located in SUR1, and many in the NBDs (62).

In general, disease severity correlates with the extent of nucleotide response impairment (30, 40, 42, 58). Moreover, mutations that impair MgADP response also tend to impair channel response to diazoxide (40, 58), which acts by stabilizing SUR1 in MgADP/MgATP stimulated conformation (23). As diazoxide is the only K_{ATP}-targeting drug currently available to treat CHI, patients with mutations that cause severe impairment of MgADP response often fail to respond to diazoxide and require alternative interventions (63). Less common are mutations which reduce the open probability of the channel, for example Kir6.2 mutations that reduce channel response to membrane PIP_2 (64) or render the current unstable by disrupting Kir6.2 subunit-subunit interactions (65) (Figure 3A). Channels with these mutations generally remain responsive to MgADP and diazoxide (64, 65).

In diffuse K_{ATP}-HI, gating mutations often follow a dominant inheritance pattern (Figure 3B). Because each K_{ATP} channel contains four Kir6.2 and four SUR1 subunits, heterozygous mutations presumably generate a mixed channel population containing 0, 1, 2, 3, or 4 mutant subunits at a ratio of 1:4:6:4:1, with the gating defect more pronounced as the number of mutant subunit increases. A subunit with a mutation that affects channel gating, but that is able to co-assemble and traffic to the cell surface, would have its gating defect manifested in the total surface channel population. The extent of the MgADP and diazoxide gating defect has been correlated with disease severity and clinical response to diazoxide in CHI children with dominant SUR1 mutations (40, 58). Thus, response of mutant channels to MgADP and diazoxide in electrophysiology experiments may be helpful in predicting disease phenotype and clinical response to diazoxide.

Mutations disrupting channel folding, assembly and trafficking

Many CHI mutations cause improper channel folding, assembly, and trafficking to the plasma membrane (53). The consequent reduction in K_{ATP} currents leads to a state of persistent β-cell membrane depolarization and uncontrolled insulin secretion. Translation, folding, and assembly of K_{ATP} channel subunits occur in the endoplasmic reticulum (ER) membrane. Upon correct assembly into a hetero-octameric complex, channels exit the ER and traffic to the Golgi. In the Golgi apparatus, SUR1 becomes complex glycosylated at its two N-linked glycosylation sites, giving rise to a mature form that migrates slower on SDS gel compared to the core-glycosylated immature form found in the ER (66, 67). The appearance and intensity of the mature SUR1 band can be used to infer channel proteins that are competent to traffic to the plasma membrane. Conversely, the absence or weakened mature SUR1 band indicates folding/assembly/trafficking defects (Figure 3C). More direct assessment of K_{ATP} channel surface expression can be achieved by surface immunofluorescence staining, surface biotinylation followed by pulldown of biotinylated protein and immunoblotting with anti-SUR1 antibody, or by electrophysiological measurement of current density (68–71).

To date, about fifty missense/indel SUR1 and Kir6.2 mutations have been reported to impair K_{ATP} channel expression at the cell surface, collectively referred to as trafficking mutations (29, 30, 53, 70, 72, 73). The extent of the impairment varies, with some mutations completely abolishing the mature SUR1 band and others reducing but not eliminating mature SUR1 (71, 74). A prominent example of the former is SUR1ΔF1388 (68), a common recessive mutation found in the Ashkenazi Jewish population (75). The mutant protein is retained in the ER, unable to reach the mature complex-glycosylated state likely because it is misfolded (68), akin to the most prevalent cystic fibrosis-causing mutation CFTRΔF508 (76). Although trafficking mutations are mapped throughout the SUR1 and Kir6.2 proteins, a high percentage are found in the first transmembrane domain of SUR1, called TMD0, and the first transmembrane helix (TM1) of Kir6.2 (53, 72). Recent high resolution 3D structures of the channel complex show that SUR1-TMD0 makes direct contact with Kir6.2-TM1, forming the primary anchor between the two subunits (77–79) (Figure 4). Mutations in these domains likely interfere with channel assembly, and thereby the trafficking of channels to the plasma membrane.

In contrast to gating mutations, trafficking mutations reported to date have been associated with recessive CHI and do not respond to diazoxide treatment (15). The recessive nature of these mutations implies that under heterozygous condition the mutant allele may be too defective or is outcompeted by the wild-type allele to form a channel complex. Indeed, some trafficking mutations have been shown to reduce subunit association, and some have been shown to cause rapid degradation of channel proteins (72, 80). This leaves the wild-type subunit to assemble and form normal functional channels, resulting in a haplosufficiency phenotype (Figure 3D). It is possible that certain mutations that cause only mild trafficking defects such that mutant proteins are still able to assemble with the wild-type allele and reach the cell surface, can remain undetected in heterozygous conditions where the mutation does not disrupt gating sufficiently to cause disease. In this regard, it is interesting to note that many heterozygous K_{ATP} mutations identified in neonatal diabetes have been shown to cause trafficking defects in addition to gain-of-function gating defects. These mutant proteins are still able to form complex with wild-type subunit and exert their gain-of-function gating effect to cause dominant disease (81–83).

Treatment of K_{ATP}-HI: Challenges and opportunities

Early diagnosis and effective treatment are critical to prevent serious neurocognitive impairments that dramatically impact CHI patients and their families due to severe associated morbidity with lifelong disability (3, 57). For focal K_{ATP}-HI, complete surgical removal of the lesion often leads to a cure (33). However, treatment for diffuse K_{ATP}-HI remains challenging. Current mainstay treatments include diazoxide, somatostatin analogues, continuous enteral feedings/dextrose, and surgery (3, 57). Diazoxide treatment would be preferred if patients have β-cell K_{ATP} channels that have

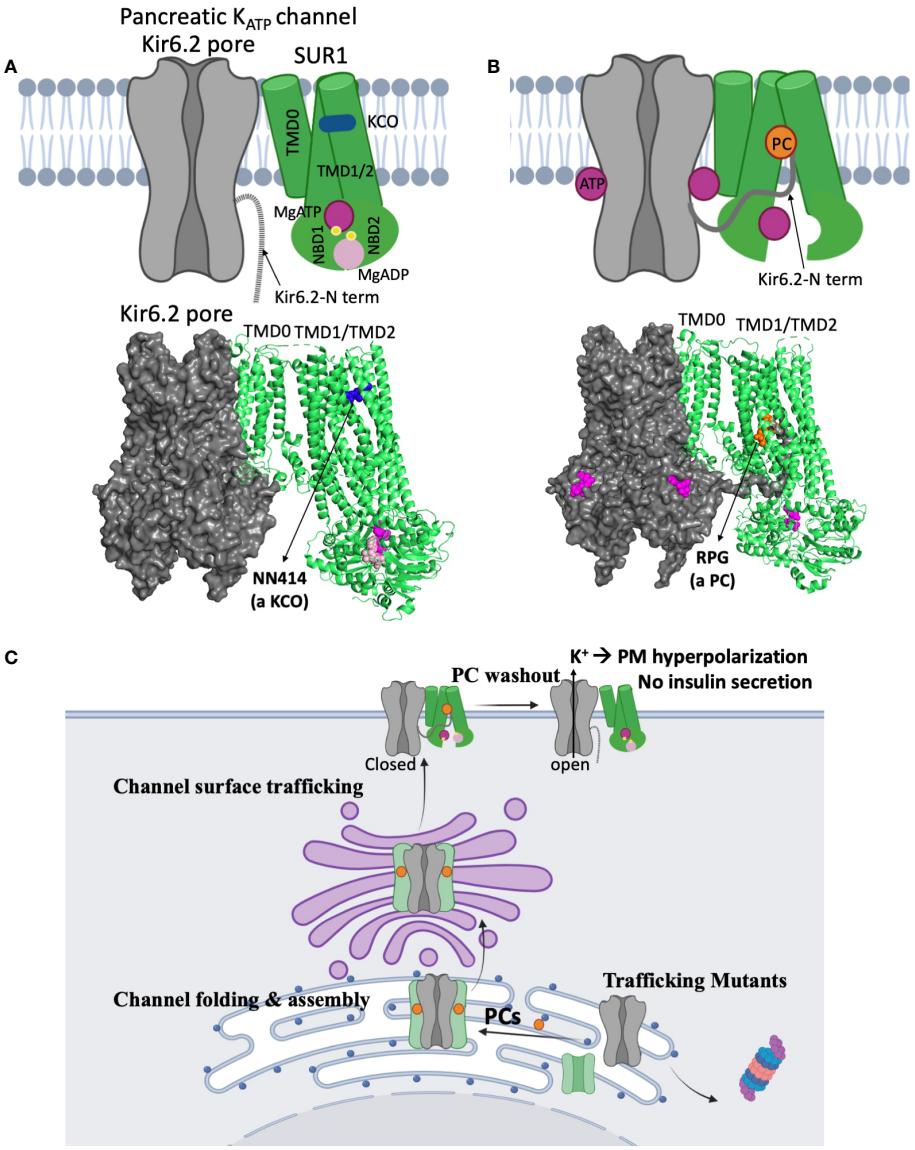

FIGURE 4
Structural insights on the mechanisms of K_{ATP} channel pharmacological modulators. **(A)** A cartoon model (*top*) illustrating the mechanism by which potassium channel openers (KCOs) stimulate channel activity, based on a recent structure of a channel bound to MgATP/MgADP and a KCO NN414 shown below (PDB ID: 7W4O; only one SUR1 subunit shown for clarity). In the structure, the SUR1 NBD1 and NBD2 are bound to MgATP and MgADP respectively and dimerized, a conformation that stimulates channel activity. NN414, which binds at a transmembrane domain pocket further stabilizes channels in the SUR1-NBDs dimerized, activated conformation to potentiate channel opening. By inference, diazoxide, a KCO used to treat some CHI patients, promotes channel opening *via* a similar mechanism. **(B)** A cartoon (*top*) showing how K_{ATP} channel inhibitors work as pharmacological chaperones (PC) based on cryoEM structures. A channel structure determined in the presence of ATP and a PC repaglinide is shown at the bottom (PDB ID: 7U1S). The structure shows that the PC (orange circle), binds in a transmembrane pocket in SUR1 formed by helices from TMD1 and TMD2. The Kir6.2 N-terminus is in a cavity formed by the two transmembrane helix bundles (TMD1/2) above the two NBDs, and is adjacent to the bound PC. This stabilizes the interaction between the N-terminal domain of Kir6.2 and SUR1 to facilitate the formation of mature hetero-octameric complex of the mutant channel. However, in this conformation the NBDs of SUR1 are separate, unable to respond to MgADP stimulation upon glucose deprivation. This explains why K_{ATP} PCs also inhibit channel activity. **(C)** A cartoon showing a proposed mechanism of how reversible inhibitor PCs can enhance channel surface expression without permanently compromising channel function. Inhibitor PCs bind to the mutant channel subunits and enhance the interaction between SUR1 central cavity and Kir6.2 N-terminus to promote mutant channel assembly and surface trafficking. Reversible inhibitor PCs are released upon washout after mutant channels are rescued to the surface, allowing the channels to open under low blood glucose conditions and inhibit insulin secretion.

sufficient response to the drug. However, many patients do not respond to diazoxide. Even with patients who do respond to diazoxide, there is often significant side effects (3, 11, 84). Currently, diazoxide is the only K_{ATP} channel opener approved in the US and Europe. Diazoxide not only activates pancreatic K_{ATP} channels but also vascular K_{ATP} channels, causing excess hair growth and other cardiovascular complications resembling Cantú syndrome patients carrying gain of function mutations in vascular K_{ATP} channels (85). Continuous feeding/dextrose infusion can restore normal glycemia, but is a heavy burden on caretakers and

patients and is associated with many complications including rapid weight gain and food aversion (3). For patients with diffuse disease and unresponsive to diazoxide and somatostatin analogues, pancreatectomy is often performed to correct the life-threatening hypoglycemia. This leads to insulin dependency later in life and digestive complications from removal of exocrine tissues (86).

With better understanding of the molecular mechanism underlying insulin secretion regulation and CHI, off label use of other drugs, such as somatostatin analogues including longer-acting octreotide (sandostatin LP, monthly injections rather than daily), glucagon, GLP-1 receptor antagonists (exendin 9-39), mTOR inhibitors (sirolimus), the calcium channel blocker nifedipine, and anti-insulin receptor antibody have been considered for CHI patients who are unresponsive to the maximum dose of diazoxide (87). However, these alternative treatments have limited success or are still in clinical trials, and do not target the root causes of K_{ATP} dysfunction in diazoxide-unresponsive diffuse CHI, namely, severe MgADP/diazoxide response defects or impaired channel trafficking to the plasma membrane. Overcoming these limitations requires better understanding of the structural mechanisms underlying channel function, dysfunction, and pharmacology.

K_{ATP} channel structures and implications for K_{ATP}-HI

A major advance in K_{ATP} channel research in recent years is our ability to visualize 3D channel structures at near atomic resolution. Using single-particle cryogenic electron microscopy (cryoEM), structures of pancreatic K_{ATP} bound to various inhibitors and activators have been determined (53, 56, 77–79, 88–91). These structures not only reveal the binding sites of key physiological and pharmacological ligands of K_{ATP} channels but also provide insights to the mechanisms of how ligands regulate channel assembly and function (17). The knowledge is of great value in future efforts to expand K_{ATP} channel pharmacology and overcome current therapeutic challenges.

Of particular interest are recent studies showing the pancreatic, cardiac, and vascular K_{ATP} channel SUR subunits, SUR1, SUR2A, and SUR2B, respectively, bound to their selective activators, NN414 (for SUR1) and P1075 or levcromakalim (for SUR2A and SUR2B) (56, 92). In the structure of SUR1 bound to NN414, NN414 sits in a transmembrane pocket that is stabilized by dimerization of the MgADP/MgATP bound NBDs of SUR1 (Figure 4A). A similar binding location for P1075 or levcromakalim is observed in SUR2A/2B. Although no diazoxide bound structure is yet available, diazoxide most likely binds to the same pocket. The structures help to explain why the effect of diazoxide requires MgADP/MgATP and why mutations that disrupt MgADP response also impair diazoxide response. Of note, NN414 stimulates K_{ATP} channel activity like diazoxide, but is more potent and selective for SUR1 containing pancreatic K_{ATP} channels (93, 94). NN414 was previously tested in clinical trials for type 2 diabetes based on the idea that opening of pancreatic K_{ATP} will allow β-cell rest and restore insulin secretion; however, the trial was stopped due to concerns over elevated liver enzymes (94). Whether NN414 can be used to treat CHI at concentrations that do not elicit hepatic or Cantú-like side effects remains untested. Regardless, the structures of different SUR isoforms bound to different openers (17, 19) provide a framework for rational drug design with improved specificity for pancreatic K_{ATP} channels without undesirable side effects to expand the medical options for CHI.

A significant number of K_{ATP} gating mutations result in channels with little or no MgADP and diazoxide response (30, 40, 58). For these mutations, drugs that open channels independent of the stimulatory effect of MgADP on SUR1 may be explored. For example, recent channel structures showing an open Kir6.2 pore conformation (56, 89) could be used to search for compounds, *via* virtual screening, that may stabilize the channel in an open state.

Lastly, mutations which impair channel expression at the cell surface also require novel approaches. Here, pharmacological chaperones, small molecules that bind to K_{ATP} channel proteins to facilitate biogenesis, correct misfolding, and restore full or partial function of the affected channels represents a promising approach (53, 95). This approach has been highly successful in cystic fibrosis, a disease caused by mutations in the CFTR gene. CFTR is a chloride channel that shares structural similarity with SUR1 (96), the regulatory subunit of K_{ATP} channels. The most common cystic fibrosis-causing mutation is ΔF508, which impairs folding, and thereby surface expression of the protein (76). Several small molecules that correct the folding and trafficking defect of mutant CFTR (lumacaftor, tezacaftor, and elexacaftor) have been recently approved for clinical use (76, 97, 98).

Sulfonylureas were the first reported K_{ATP} pharmacological chaperones (99). These antidiabetics, which inhibit K_{ATP} channels and stimulate insulin secretion, include the high affinity sulfonylurea glibenclamide and the low affinity sulfonylurea tolbutamide. Subsequently, glinides, a second class of K_{ATP} inhibiting antidiabetics including repaglinide, was found to have similar pharmacological chaperone effects (100). More recently, carbamazepine, an anticonvulsant known to block voltage-gated sodium channels was also discovered as a K_{ATP} pharmacological chaperone (69). Interestingly, these compounds are all K_{ATP} channel inhibitors (69, 101). Moreover, each of these K_{ATP} channel inhibitors are effective for only a subset of trafficking mutations. In particular, they promote trafficking of channels harboring mutations in the TMD0 domain of SUR1, which is the primary domain that interacts with Kir6.2 (69, 100), suggesting drug binding corrects trafficking defects by facilitating subunit assembly (102). By examining cryoEM structures of channels bound to glibenclamide, repaglinide, or carbamazepine, it was discovered that the distal N-terminus of Kir6.2 cooperates with SUR1 for drug binding, and drug binding in turn stabilizes Kir6.2-N terminus interaction with SUR1 (103) (Figure 4B). Thus, these drugs likely exert their channel chaperoning and inhibition effects *via* the same mechanism (Figure 4B), i.e. by stabilizing the Kir6.2 N-terminus in the SUR1 ABC core cavity. By contrast, channel openers such as diazoxide and NN414, which stabilize SUR1-NBD dimerization and exclude Kir6.2 N-terminus from the SUR1 ABC core cavity (Figure 4A), diminish Kir6.2-SUR1 interactions and have been shown to lack chaperoning activity (74).

While promising, translation of the above basic science finding to CHI treatment has a number of challenges. First, all K_{ATP} pharmacological chaperones reported to date are inhibitors. The

most potent chaperones like glibenclamide and repaglinide are also the most potent inhibitors. The nearly irreversible inhibition of channels by these high affinity inhibitors precludes functional recovery of mutant channels rescued to the cell surface (99). To date, tolbutamide is by far the most reversible inhibitor that is also effective in rescuing mutant channels to the cell surface, albeit at significantly higher concentrations (99). Tolbutamide, a first-generation sulfonylurea which has been around since the 1950s, has largely been replaced with other oral hypoglycemics and is no longer available in the US. While early randomized trials associated its use with increased cardiovascular and all-cause mortality, the increased cardiovascular and all-cause mortality was not statistically significant (104). Off label use of tolbutamide as a potential pharmacologic therapy for CHI patients with diffuse disease and K_{ATP} trafficking mutations is an intriguing possibility (Figure 4C). Secondly, some trafficking mutations also disrupt channel gating. For example, two CHI-causing SUR1 trafficking mutations, R74W and E128K, also render channels less sensitive to ATP inhibition such that upon pharmacological rescue to the plasma membrane mutant channels cause membrane hyperpolarization and decreased glucose-stimulated insulin secretion in cultured insulinoma cells that resemble neonatal diabetes mutation phenotypes (105). Thirdly, not all trafficking mutations respond to the K_{ATP} pharmacological chaperones identified to date (71, 99). These mutations, largely located outside TMD0 of SUR1, likely cause severe misfolding such that mutant proteins are triaged for degradation (99, 106). Additional drug screening studies such as those done for CFTRΔF508 will be required to overcome defects caused by such mutations. Finally, there is currently no animal models to test the *in vivo* feasibility of pharmacological chaperone therapy. Recent development of induced pluripotent stem cells (iPSCs) derived from a CHI patient carrying a homozygous trafficking mutation SUR1-V187D (107) could serve as an intermediate experimental model to test the effect of reversible K_{ATP} pharmacological chaperones such as tolbutamide.

Concluding remarks

Much progress has been made in the diagnosis and management of K_{ATP}-HI since the first report linking K_{ATP} channel gene mutations to CHI (108). There are now hundreds of mutations that have been identified, and there has been steady progress in our understanding of genotype-phenotype correlation, mutation mechanisms, and drug response. The growing knowledge base facilitates rapid diagnosis and treatment. Despite the progress, timely and accurate molecular diagnosis of patients carrying variants of unknown significance, and treatment of diazoxide-unresponsive diffuse disease caused by severe gating and trafficking mutations remain challenging. With recent rapid technical advances in gene sequencing, bioinformatics, channel structure determination, machine-learning based drug design, and gene therapy, there is great optimism that new and personalized therapies for K_{ATP}-HI will become a reality in the not-too-distant future.

Author contributions

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. Giri D, Hawton K, Senniappan S. Congenital hyperinsulinism: recent updates on molecular mechanisms, diagnosis and management. *J Pediatr Endocrinol Metab* (2022) 35(3):279–96. doi: 10.1515/jpem-2021-0369

2. Thornton PS, Stanley CA, De Leon DD. Congenital hyperinsulinism: An historical perspective. *Horm Res Paediatr* (2022) 95(6):631–7. doi: 10.1159/000526442

3. Banerjee I, Raskin J, Arnoux J.B, Leon De D.D, Weinzimer S.A, 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

4. de Lonlay P, Fournet JC, Touati G, Groos MS, Martin D, Sevin C, et al. Heterogeneity of persistent hyperinsulinaemic hypoglycaemia. a series of 175 cases. *Eur J Pediatr* (2002) 161(1):37–48. doi: 10.1007/s004310100847

5. Mc QI. Idiopathic spontaneously occurring hypoglycemia in infants; clinical significance of problem and treatment. *AMA Am J Dis Child* (1954) 87(4):399–428.

6. Galcheva S, Demirbilek H, Al-Khawaga S, Hussain K. The genetic and molecular mechanisms of congenital hyperinsulinism. *Front Endocrinol (Lausanne)* (2019) 10:111. doi: 10.3389/fendo.2019.00111

7. 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

8. Yakovac WC, Baker L, Hummeler K. Beta cell nesidioblastosis in idiopathic hypoglycemia of infancy. *J Pediatr* (1971) 79(2):226–31. doi: 10.1016/S0022-3476(71)80105-1

9. Jaffe R, Hashida Y, Yunis EJ. Pancreatic pathology in hyperinsulinemic hypoglycemia of infancy. *Lab Invest* (1980) 42(3):356–65.

10. Rahier J, Wallon J, Henquin JC. Cell populations in the endocrine pancreas of human neonates and infants. *Diabetologia* (1981) 20(5):540–6. doi: 10.1007/BF00252762

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

12. 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

13. Pasquini TLS, Mesfin M, Schmitt J, Raskin J. Global registries in congenital hyperinsulinism. *Front Endocrinol (Lausanne)* (2022) 13:876903. doi: 10.3389/fendo.2022.876903

14. Amaratunga SA, Tayeb TH, Dusatkova P, Pruhova S, Lebl J. Invaluable role of consanguinity in providing insight into paediatric endocrine conditions: Lessons learnt from congenital hyperinsulinism, monogenic diabetes, and short stature. *Horm Res Paediatr* (2022) 95(1):1–11. doi: 10.1159/000521210

15. 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

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

17. Driggers CM, Shyng SL. Mechanistic insights on KATP channel regulation from cryo-EM structures. *J Gen Physiol* (2023) 155(1):e202113046. doi: 10.1085/jgp.202113046

18. Wu JX, Ding D, Chen L. The emerging structural pharmacology of ATP-sensitive potassium channels. *Mol Pharmacol* (2022) 102(5):234–9. doi: 10.1124/molpharm.122.000570

19. Martin GM, Patton BL, Shyng SL. K(ATP) channels in focus: Progress toward a structural understanding of ligand regulation. *Curr Opin Struct Biol* (2023) 79:102541. doi: 10.1016/j.sbi.2023.102541

20. Aguilar-Bryan L, Bryan J. Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr Rev* (1999) 20(2):101–35. doi: 10.1210/edrv.20.2.0361

21. Ashcroft FM. K(ATP) channels and insulin secretion: a key role in health and disease. *Biochem Soc Trans* (2006) 34(Pt 2):243–6. doi: 10.1042/BST0340243

22. Inagaki N, Gonoi T, Clement JPT, Namba N, Inazawa J, Gonzalez G, et al. Reconstitution of IKATP: An inward rectifier subunit plus the sulfonylurea receptor. *Science* (1995) 270(5239):1166–70. doi: 10.1126/science.270.5239.1166

23. Nichols CG. KATP channels as molecular sensors of cellular metabolism. *Nature* (2006) 440(7083):470–6. doi: 10.1038/nature04711

24. Aguilar-Bryan L, Nichols C.G, Wechsler S.W, Clement J. P. t, Boyd A.E 3rd, Gonzalez G, et al. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* (1995) 268(5209):423–6. doi: 10.1126/science.7716547

25. Ashcroft FM. The walter b. cannon physiology in perspective lecture, 2007. ATP-sensitive k+ channels and disease: from molecule to malady. *Am J Physiol Endocrinol Metab* (2007) 293(4):E880–9. doi: 10.1152/ajpendo.00348.2007

26. Huopio H, Shyng SL, Otonkoski T, Nichols CG. K(ATP) channels and insulin secretion disorders. *Am J Physiol Endocrinol Metab* (2002) 283(2):E207–16. doi: 10.1152/ajpendo.00047.2002

27. Sharma N, Crane A, Gonzalez G, Bryan J, Aguilar-Bryan L. Familial hyperinsulinism and pancreatic beta-cell ATP-sensitive potassium channels. *Kidney Int* (2000) 57(3):803–8. doi: 10.1046/j.1523-1755.2000.00918.x

28. 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

29. 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

30. Boodhansingh KE, Kandasamy B, Mitteer L, Givler S, Leon De DD, Shyng SL, et al. Novel dominant K(ATP) channel mutations in infants with congenital hyperinsulinism: Validation by in vitro expression studies and in vivo carrier phenotyping. *Am J Med Genet A* (2019) 179(11):2214–27. doi: 10.1002/ajmg.a.61335

31. Saint-Martin C, Miere Cauchois-Le M, Rex E, Soukarieh O, Arnoux JB, Buratti J, et al. Functional characterization of ABCC8 variants of unknown significance based on bioinformatics predictions, splicing assays, and protein analyses: Benefits for the accurate diagnosis of congenital hyperinsulinism. *Hum Mutat* (2021) 42(4):408–20. doi: 10.1002/humu.24164

32. Bellanne-Chantelot C, Saint-Martin C, Ribeiro MJ, Vaury C, Verkarre V, Arnoux JB, et al. ABCC8 and KCNJ11 molecular spectrum of 109 patients with diazoxide-unresponsive congenital hyperinsulinism. *J Med Genet* (2010) 47(11):752–9. doi: 10.1136/jmg.2009.075416

33. Pizzoferro M, Masselli G, Maiorana A, Casciani E, Sollaku S, Dionisi-Vici C, et al. PET/CT in congenital hyperinsulinism: transforming patient's lives by molecular hybrid imaging. *Am J Nucl Med Mol Imaging* (2022) 12(2):44–53.

34. Rasmussen AG, Melikian M, Globa E, Detlefsen S, Rasmussen L, Petersen H, et al. The difficult management of persistent, non-focal congenital hyperinsulinism: A retrospective review from a single, tertiary center. *Pediatr Diabetes* (2020) 21(3):441–55. doi: 10.1111/pedi.12989

35. Sait H, Sharma L, Dabadghao P, Phadke SR. Congenital hyperinsulinemia of infancy: Role of molecular testing in management and genetic counseling. *Indian J Pediatr* (2022) 89(4):395–8. doi: 10.1007/s12098-021-04014-x

36. 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

37. Ismail D, Smith VV, Lonlay P, Ribeiro MJ, Rahier J, Blankenstein O, et al. Familial focal congenital hyperinsulinism. *J Clin Endocrinol Metab* (2011) 96(1):24–8. doi: 10.1210/jc.2010-1524

38. Faletra F, Snider K, Shyng SL, Bruno I, Athanasakis E, Gasparini P, et al. Co-Inheritance of two ABCC8 mutations causing an unresponsive congenital hyperinsulinism: clinical and functional characterization of two novel ABCC8 mutations. *Gene* (2013) 516(1):122–5. doi: 10.1016/j.gene.2012.12.055

39. Flanagan SE, Kapoor RR, Banerjee I, Hall C, Smith VV, Hussain K, et al. Dominantly acting ABCC8 mutations in patients with medically unresponsive hyperinsulinaemic hypoglycaemia. *Clin Genet* (2011) 79(6):582–7. doi: 10.1111/j.1399-0004.2010.01476.x

40. Macmullen CM, Zhou Q, Snider KE, Tewson PH, Becker SA, Aziz AR, et al. Diazoxide-unresponsive congenital hyperinsulinism in children with dominant mutations of the beta-cell sulfonylurea receptor SUR1. *Diabetes* (2011) 60(6):1797–804. doi: 10.2337/db10-1631

41. Nessa A, Aziz QH, Thomas AM, Harmer SC, Tinker A, Hussain K. Molecular mechanisms of congenital hyperinsulinism due to autosomal dominant mutations in ABCC8. *Hum Mol Genet* (2015) 24(18):5142–53. doi: 10.1093/hmg/ddv233

42. Pinney SE, MacMullen C, Becker S, Lin YW, Hanna C, Thornton P, et al. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. *J Clin Invest* (2008) 118(8):2877–86. doi: 10.1172/JCI35414

43. Huopio H, Reimann F, Ashfield R, Komulainen J, Lenko HL, 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

44. Perge K, Nicolino M. Variable phenotypes of individual and family monogenic cases with hyperinsulinism and diabetes: a systematic review. *Rev Endocr Metab Disord* (2022) 23(5):1063–78. doi: 10.1007/s11154-022-09749-2

45. Shemer R, Avnon Ziv C, Laiba E, Zhou Q, Gay J, Tunovsky-Babaey S, et al. Relative expression of a dominant mutated ABCC8 allele determines the clinical manifestation of congenital hyperinsulinism. *Diabetes* (2012) 61(1):258–63. doi: 10.2337/db11-0984

46. Kim JW, Seghers V, Cho JH, Kang Y, Kim S, Ryu Y, et al. Transactivation of the mouse sulfonylurea receptor I gene by BETA2/NeuroD. *Mol Endocrinol* (2002) 16 (5):1097–107. doi: 10.1210/mend.16.5.0934

47. Moritz W, Leech CA, Ferrer J, Habener JF. Regulated expression of adenosine triphosphate-sensitive potassium channel subunits in pancreatic beta-cells. *Endocrinology* (2001) 142(1):129–38. doi: 10.1210/endo.142.1.7885

48. 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

49. Kiff S, Babb C, Guemes M, Dastamani A, Gilbert C, Flanagan SE, et al. Partial diazoxide responsiveness in a neonate with hyperinsulinism due to homozygous ABCC8 mutation. *Endocrinol Diabetes Metab Case Rep* (2019) 2019:18–0120. doi: 10.1530/EDM-18-0120

50. Reyes Diaz JV, Jin Y, Garber K, Cossen KM, Li Y, Jin P, et al. A homozygous exonic variant leading to exon skipping in ABCC8 as the cause of severe congenital hyperinsulinism. *Am J Med Genet A* (2022) 188(8):2429–33. doi: 10.1002/ajmg.a.62843

51. Takasawa K, Miyakawa Y, Saito Y, Adachi E, Shidei T, Sutani A, et al. Marked clinical heterogeneity in congenital hyperinsulinism due to a novel homozygous ABCC8 mutation. *Clin Endocrinol (Oxf)* (2021) 94(6):940–8. doi: 10.1111/cen.14443

52. Liu Y, Yang Q, Zhao F. Synonymous but not silent: The codon usage code for gene expression and protein folding. *Annu Rev Biochem* (2021) 90:375–401. doi: 10.1146/annurev-biochem-071320-112701

53. Martin GM, Sung MW, Shyng SL. Pharmacological chaperones of ATP-sensitive potassium channels: Mechanistic insight from cryoEM structures. *Mol Cell Endocrinol* (2020) 502:110667. doi: 10.1016/j.mce.2019.110667

54. Kandasamy B, Shyng SL. Methods for characterizing disease-associated ATP-sensitive potassium channel mutations. *Methods Mol Biol* (2018) 1684:85–104. doi: 10.1007/978-1-4939-7362-0_8

55. Thornton PS, MacMullen C, Ganguly A, Ruchelli E, Steinkrauss L, Crane A, et al. Clinical and molecular characterization of a dominant form of congenital hyperinsulinism caused by a mutation in the high-affinity sulfonylurea receptor. *Diabetes* (2003) 52(9):2403–10. doi: 10.2337/diabetes.52.9.2403

56. Wang M, Wu JX, Ding D, Chen L. Structural insights into the mechanism of pancreatic K(ATP) channel regulation by nucleotides. *Nat Commun* (2022) 13(1):2770. doi: 10.1038/s41467-022-30430-4

57. Thornton PS. Recent updates in the management of infants and children with hyperinsulinism. *Curr Opin Pediatr* (2021) 33(4):424–9. doi: 10.1097/MOP.0000000000001022

58. Saint-Martin C, Zhou Q, Martin GM, Vaury C, Leroy G, Arnoux JB, et al. Monoallelic ABCC8 mutations are a common cause of diazoxide-unresponsive diffuse form of congenital hyperinsulinism. *Clin Genet* (2015) 87(5):448–54. doi: 10.1111/cge.12428

59. Baukrowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, et al. PIP2 and PIP as determinants for ATP inhibition of KATP channels. *Science* (1998) 282 (5391):1141–4. doi: 10.1126/science.282.5391.1141

60. Shyng SL, Nichols CG. Membrane phospholipid control of nucleotide sensitivity of KATP channels. *Science* (1998) 282(5391):1138–41. doi: 10.1126/science.282.5391.1138

61. Nichols CG, Shyng SL, Nestorowicz A, Glaser B, Clement JPT, Gonzalez G, et al. Adenosine diphosphate as an intracellular regulator of insulin secretion. *Science* (1996) 272(5269):1785–7. doi: 10.1126/science.272.5269.1785

62. Shyng SL, Ferrigni T, Shepard JB, Nestorowicz A, Glaser B, Permutt MA, et al. Functional analyses of novel mutations in the sulfonylurea receptor 1 associated with persistent hyperinsulinemic hypoglycemia of infancy. *Diabetes* (1998) 47(7):1145–51. doi: 10.2337/diabetes.47.7.1145

63. Worth C, Yau D, Estebanez Salomon M, E, Cosgrove K, Dunne M, et al. Complexities in the medical management of hypoglycaemia due to congenital hyperinsulinism. *Clin Endocrinol (Oxf)* (2020) 92(5):387–95. doi: 10.1111/cen.14152

64. Lin YW, MacMullen C, Ganguly A, Stanley CA, Shyng SL. A novel KCNJ11 mutation associated with congenital hyperinsulinism reduces the intrinsic open probability of beta-cell ATP-sensitive potassium channels. *J Biol Chem* (2006) 281 (5):3006–12. doi: 10.1074/jbc.M511875200

65. Lin YW, Bushman JD, Yan FF, Haidar S, MacMullen C, Ganguly A, et al. Destabilization of ATP-sensitive potassium channel activity by novel KCNJ11 mutations identified in congenital hyperinsulinism. *J Biol Chem* (2008) 283 (14):9146–56. doi: 10.1074/jbc.M708798200

66. Raab-Graham KF, Cirilo LJ, Boettcher AA, Radeke CM, Vandenberg CA. Membrane topology of the amino-terminal region of the sulfonylurea receptor. *J Biol Chem* (1999) 274(41):29122–9. doi: 10.1074/jbc.274.41.29122

67. Zerangue N, Schwappach B, Jan YN, Jan LY. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron* (1999) 22(3):537–48. doi: 10.1016/S0896-6273(00)80708-4

68. Cartier EA, Conti LR, Vandenberg CA, Shyng SL. Defective trafficking and function of KATP channels caused by a sulfonylurea receptor 1 mutation associated with persistent hyperinsulinemic hypoglycemia of infancy. *Proc Natl Acad Sci U.S.A.* (2001) 98(5):2882–7. doi: 10.1073/pnas.051499698

69. Chen PC, Olson EM, Zhou Q, Kryukova Y, Sampson HM, Thomas DY, et al. Carbamazepine as a novel small molecule corrector of trafficking-impaired ATP-sensitive potassium channels identified in congenital hyperinsulinism. *J Biol Chem* (2013) 288(29):20942–54. doi: 10.1074/jbc.M113.470948

70. Reimann F, Huopio H, Dabrowski M, Proks P, Gribble FM, Laakso M, et al. Characterisation of new KATP-channel mutations associated with congenital hyperinsulinism in the Finnish population. *Diabetologia* (2003) 46(2):241–9. doi: 10.1007/s00125-002-1014-3

71. Yan FF, Lin YW, MacMullen C, Ganguly A, Stanley CA, Shyng SL, et al. Congenital hyperinsulinism associated ABCC8 mutations that cause defective trafficking of ATP-sensitive k+ channels: identification and rescue. *Diabetes* (2007) 56(9):2339–48. doi: 10.2337/db07-0150

72. Crane A, Aguilar-Bryan L. Assembly, maturation, and turnover of K(ATP) channel subunits. *J Biol Chem* (2004) 279(10):9080–90. doi: 10.1074/jbc.M311079200

73. Fukuda Y, Aguilar-Bryan L, Vaxillaire M, Dechaume A, Wang Y, Dean M, et al. Conserved intramolecular disulfide bond is critical to trafficking and fate of ATP-binding cassette (ABC) transporters ABCB6 and sulfonylurea receptor 1 (SUR1)/ABCC8. *J Biol Chem* (2011) 286(10):8481–92. doi: 10.1074/jbc.M110.174516

74. Martin GM, Rex EA, Devaraneni P, Denton JS, Boodhansingh KE, DeLeon DD, et al. Pharmacological correction of trafficking defects in ATP-sensitive potassium channels caused by sulfonylurea receptor 1 mutations. *J Biol Chem* (2016) 291 (42):21971–83. doi: 10.1074/jbc.M116.749366

75. Nestorowicz A, Glaser B, Wilson BA, Shyng SL, Nichols CG, Stanley CA, et al. Genetic heterogeneity in familial hyperinsulinism. *Hum Mol Genet* (1998) 7(7):1119–28. doi: 10.1093/hmg/7.7.1119

76. Brusa I, Sondo E, Falchi F, Pedemonte N, Roberti M, Cavalli A, et al. Proteostasis regulators in cystic fibrosis: Current development and future perspectives. *J Med Chem* (2022) 65(7):5212–43. doi: 10.1021/acs.jmedchem.1c01897

77. Lee KPK, Chen J, MacKinnon R. Molecular structure of human KATP in complex with ATP and ADP. *Elife* (2017) 6:e32481. doi: 10.7554/eLife.32481

78. Li N, Wu JX, Ding D, Cheng J, Gao N, Chen L, et al. Structure of a pancreatic ATP-sensitive potassium channel. *Cell* (2017) 168(1-2):101–110 e10. doi: 10.1016/j.cell.2016.12.028

79. Martin GM, Yoshioka C, Rex EA, Fay JF, Xie Q, Whorton MR, et al. Cryo-EM structure of the ATP-sensitive potassium channel illuminates mechanisms of assembly and gating. *Elife* (2017) 6:e24149. doi: 10.7554/eLife.24149

80. Chan KW, Zhang H, Logothetis DE. N-terminal transmembrane domain of the SUR controls trafficking and gating of Kir6 channel subunits. *EMBO J* (2003) 22 (15):3833–43. doi: 10.1093/emboj/cdg376

81. Lin CW, Lin YW, Yan FF, Casey J, Kochhar M, Pratt EB, et al. Kir6.2 mutations associated with neonatal diabetes reduce expression of ATP-sensitive k+ channels: implications in disease mechanism and sulfonylurea therapy. *Diabetes* (2006) 55 (6):1738–46. doi: 10.2337/db05-1571

82. Lin YW, Li A, Grasso V, Battaglia D, Crino A, Colombo C, et al. Functional characterization of a novel KCNJ11 in frame mutation-deletion associated with infancy-onset diabetes and a mild form of intermediate DEND: a battle between K (ATP) gain of channel activity and loss of channel expression. *PloS One* (2013) 8(5): e63758. doi: 10.1371/journal.pone.0063758

83. Zhou Q, Garin I, Castano L, Argente J, Munoz-Calvo MT, Nanclares Perez G, et al. Neonatal diabetes caused by mutations in sulfonylurea receptor 1: interplay between expression and mg-nucleotide gating defects of ATP-sensitive potassium channels. *J Clin Endocrinol Metab* (2010) 95(12):E473–8. doi: 10.1210/jc.2010-1231

84. Yildizdas D, Erdem S, Kucukosmanoglu O, Yilmaz M, Yuksel B. Pulmonary hypertension, heart failure and neutropenia due to diazoxide therapy. *Adv Ther* (2008) 25(5):515–9. doi: 10.1007/s12325-008-0049-3

85. Nichols CG. Personalized therapeutics for K(ATP)-dependent pathologies. *Annu Rev Pharmacol Toxicol* (2023) 63:541–63. doi: 10.1146/annurev-pharmtox-051921-123023

86. Banerjee I, Salomon-Estebanez M, Shah P, Nicholson J, Cosgrove KE, Dunne MJ, et al. Therapies and outcomes of congenital hyperinsulinism-induced hypoglycaemia. *Diabetes Med* (2019) 36(1):9–21. doi: 10.1111/dme.13823

87. De Cosio AP, Thornton P. Current and emerging agents for the treatment of hypoglycemia in patients with congenital hyperinsulinism. *Paediatr Drugs* (2019) 21 (3):123–36. doi: 10.1007/s40272-019-00334-w

88. Martin GM, Kandasamy B, DiMaio F, Yoshioka C, Shyng SL. Anti-diabetic drug binding site in a mammalian K(ATP) channel revealed by cryo-EM. *Elife* (2017) 6: e31054. doi: 10.7554/eLife.31054

89. Zhao C, MacKinnon R. Molecular structure of an open human K(ATP) channel. *Proc Natl Acad Sci U.S.A.* (2021) 118(48):e2112267118. doi: 10.1073/pnas.2112267118

90. Ding D, Wang M, Wu JX, Kang Y, Chen L. The structural basis for the binding of repaglinide to the pancreatic K(ATP) channel. *Cell Rep* (2019) 27(6):1848–1857 e4. doi: 10.1016/j.celrep.2019.04.050

91. Wu JX, Ding D, Wang M, Kang Y, Zeng X, Chen L, et al. Ligand binding and conformational changes of SUR1 subunit in pancreatic ATP-sensitive potassium channels. *Protein Cell* (2018) 9(6):553–67. doi: 10.1007/s13238-018-0530-y

92. Ding D, Wu JX, Duan X, Ma S, Lai L, Chen L, et al. Structural identification of vasodilator binding sites on the SUR2 subunit. *Nat Commun* (2022) 13(1):2675. doi: 10.1038/s41467-022-30428-y

93. 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 (Lausanne)* (2020) 11:545638. doi: 10.3389/fendo.2020.545638

94. Zdravkovic M, Kruse M, Rost KL, Moss J, Kecskes A. The effects of NN414, a SUR1/Kir6.2 selective potassium channel opener in subjects with type 2 diabetes. *Exp Clin Endocrinol Diabetes* (2007) 115(6):405–6. doi: 10.1055/s-2007-973062

95. Leidenheimer NJ, Ryder KG. Pharmacological chaperoning: a primer on mechanism and pharmacology. *Pharmacol Res* (2014) 83:10–9. doi: 10.1016/j.phrs.2014.01.005

96. Thomas C, Tampe R. Structural and mechanistic principles of ABC transporters. *Annu Rev Biochem* (2020) 89:605–36. doi: 10.1146/annurev-biochem-011520-105201

97. Fiedorczuk K, Chen J. Mechanism of CFTR correction by type I folding correctors. *Cell* (2022) 185(1):158–168 e11. doi: 10.1016/j.cell.2021.12.009

98. Lopes-Pacheco M. CFTR modulators: The changing face of cystic fibrosis in the era of precision medicine. *Front Pharmacol* (2019) 10:1662. doi: 10.3389/fphar.2019.01662

99. Yan F, Lin CW, Weisiger E, Cartier EA, Taschenberger G, Shyng SL, et al. Sulfonylureas correct trafficking defects of ATP-sensitive potassium channels caused by mutations in the sulfonylurea receptor. *J Biol Chem* (2004) 279(12):11096–105. doi: 10.1074/jbc.M312810200

100. Yan FF, Casey J, Shyng SL. Sulfonylureas correct trafficking defects of disease-causing ATP-sensitive potassium channels by binding to the channel complex. *J Biol Chem* (2006) 281(44):33403–13. doi: 10.1074/jbc.M605195200

101. Zhou Q, Chen PC, Devaraneni PK, Martin GM, Olson EM, Shyng SL, et al. Carbamazepine inhibits ATP-sensitive potassium channel activity by disrupting channel response to MgADP. *Channels (Austin)* (2014) 8(4):376–82. doi: 10.4161/chan.29117

102. Devaraneni PK, Martin GM, Olson EM, Zhou Q, Shyng SL. Structurally distinct ligands rescue biogenesis defects of the KATP channel complex via a converging mechanism. *J Biol Chem* (2015) 290(12):7980–91. doi: 10.1074/jbc.M114.634576

103. Martin GM, Sung MW, Yang Z, Innes LM, Kandasamy B, David LL, et al. Mechanism of pharmacochaperoning in a mammalian K(ATP) channel revealed by cryo-EM. *Elife* (2019) 8:e46417. doi: 10.7554/eLife.46417

104. Schwartz TB, Meinert CL. The UGDP controversy: thirty-four years of contentious ambiguity laid to rest. *Perspect Biol Med* (2004) 47(4):564–74. doi: 10.1353/pbm.2004.0071

105. Pratt EB, Yan FF, Gay JW, Stanley CA, Shyng SL. Sulfonylurea receptor 1 mutations that cause opposite insulin secretion defects with chemical chaperone exposure. *J Biol Chem* (2009) 284(12):7951–9. doi: 10.1074/jbc.M807012200

106. Yan FF, Lin CW, Cartier EA, Shyng SL. Role of ubiquitin-proteasome degradation pathway in biogenesis efficiency of beta-cell ATP-sensitive potassium channels. *Am J Physiol Cell Physiol* (2005) 289(5):C1351–9. doi: 10.1152/ajpcell.00240.2005

107. Lithovius V, Otonkoski T. Stem cell based models in congenital hyperinsulinism - perspective on practicalities and possibilities. *Front Endocrinol (Lausanne)* (2022) 13:837450. doi: 10.3389/fendo.2022.837450

108. 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

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Current understanding on pathogenesis and effective treatment of glycogen storage disease type Ib with empagliflozin: new insights coming from diabetes for its potential implications in other metabolic disorders

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Glycogen storage type Ib (GSDIb) is a rare inborn error of metabolism caused by glucose-6-phosphate transporter (G6PT, *SLC37A4*) deficiency. G6PT defect results in excessive accumulation of glycogen and fat in the liver, kidney, and intestinal mucosa and into both glycogenolysis and gluconeogenesis impairment. Clinical features include hepatomegaly, hypoglycemia, lactic acidemia, hyperuricemia, hyperlipidemia, and growth retardation. Long-term complications are liver adenoma, hepatocarcinoma, nephropathy and osteoporosis. The hallmark of GSDIb is neutropenia, with impaired neutrophil function, recurrent infections and inflammatory bowel disease. Alongside classical nutritional therapy with carbohydrates supplementation and immunological therapy with granulocyte colony-stimulating factor, the emerging role of 1,5-anhydroglucitol in the pathogenesis of neutrophil dysfunction led to repurpose empagliflozin, an inhibitor of the renal glucose transporter SGLT2: the current literature of its off-label use in GSDIb patients reports beneficial effects on neutrophil dysfunction and its clinical consequences. Surprisingly, this glucose-lowering drug ameliorated the glycemic and metabolic control in GSDIb patients. Furthermore, numerous studies from big cohorts of type 2 diabetes patients showed the efficacy of empagliflozin in reducing the cardiovascular risk, the progression of kidney disease, the NAFLD and the metabolic syndrome. Beneficial effects have also been described on peripheral neuropathy in a prediabetic rat model. Increasing evidences highlight the role of empagliflozin in regulating the cellular energy sensors SIRT1/AMPK and Akt/mTOR, which leads to improvement of mitochondrial structure and function, stimulation of autophagy, decrease of oxidative stress and suppression of inflammation. Modulation of these pathways shift the oxidative metabolism from carbohydrates to lipids oxidation and results

crucial in reducing insulin levels, insulin resistance, glucotoxicity and lipotoxicity. For its pleiotropic effects, empagliflozin appears to be a good candidate for drug repurposing also in other metabolic diseases presenting with hypoglycemia, organ damage, mitochondrial dysfunction and defective autophagy.

KEYWORDS

glycogen storage diseases, hypoglycemia, empagliflozin, neutropenia, neutrophil dysfunction, inflammatory bowel disease, autophagy

1 Introduction

Glycogen storage type Ib (GSDIb) is a rare inborn error of metabolism (prevalence 1:500,000) (1) caused by recessive mutations of glucose-6-phosphate translocase (*SLC37A4*), which encodes the glucose-6-phosphate transporter (G6PT) located in the membrane of the endoplasmic reticulum. The ubiquitously expressed G6PT transports glucose-6-phosphate (G6P) into the lumen of the endoplasmic reticulum where it can be hydrolyzed to glucose by glucose-6-phosphatase (G6PC1), expressed in liver, kidney and gut (2). A defect in this enzymatic complex affects glucose formation because both glycogenolysis and gluconeogenesis are inhibited, with negative effects on glycemic control. As in Glycogen storage disease type Ia (GSDIa), caused by biallelic variants in the *G6PC1* gene, mutations of *SLC37A4* result in excessive glycogen and fat accumulation in liver, kidney, and intestinal mucosa. Clinical features include hepatomegaly, hypoglycemia, lactic acidemia, hyperuricemia, hyperlipidemia, and growth retardation. Infants and children manifest hypoglycemia after 2-4 hours of fasting. Furthermore, patients with GSDIb present neutropenia, neutrophil dysfunction, and are prone to recurrent infections, ano-urogenital lesions, and inflammatory bowel disease (IBD) (3). Neutropenia can start at birth or later, it can be cyclic or permanent, with a variable clinical course (4, 5). Neutrophil count is impaired by a defective production or enhanced apoptosis. GSDIb neutrophils have a dysfunctional metabolism, which causes impaired chemotaxis, oxidative burst, and bactericidal activity (6).

2 Current treatment

2.1 Nutritional aspects

Dietary treatment is the cornerstone of management for patients with G6PC1 and G6PT deficiency, aimed to prevent hypoglycemia and minimize the risk of neurological outcome, such as developmental and motor delay, seizures and death. Nutritional therapy for GSDIa and GSDIb follows the same requirements, but further dietary manipulations are necessary in patients with GSDIb in case of IBD. Avoidance of fasting is the first line of treatment through frequent feeds of high carbohydrate low lipid diet, supplemented with cornstarch (CS). In the first year of life, nocturnal meals can be replaced by continuous enteral feeding through a nasogastric tube or gastrostomy. Surgical gastrostomy should be placed during granulocyte colony-stimulating factor (G-CSF) therapy to minimize the risk of local infection or failure in wound healing (3). American guidelines recommend to provide a nocturnal enteral glucose infusion rate of 8-10 mg glucose/kg/min in infancy, and 4-8 mg glucose/kg/min in children. Calories should be provided as 60–70% from carbohydrates, 10–15% from proteins (according to daily recommended intake), <30% from fats. CS dosage should be 1.6 g/kg (ideal body weight) every 3-4 hours in young children, and 1.7-2.5g/kg every 4-6 hours in older children and adults. Some adults may require a single dose of CS at bedtime to maintain glycemia >70 mg/dl (4 mmol/l) and lactate <2 mmol/l. However, starting from the above recommendations, nocturnal and diurnal feeding regimens are then individualized, based on glucose monitoring to achieve normoglycemia. Careful glycemic and metabolic monitoring should be performed in order to avoid overfeeding and overtreatment which lead to hyperinsulinemia, insulin resistance, obesity and nutrient deficiencies (3). In the case of IBD, continuous enteral feeding may be necessary, using an elemental diet with a polymeric formula (Modulen® IBD) (7, 8), with minimum CS amount necessary to achieve glycemic and metabolic control, for the aggravative effect of CS on IBD (9).

2.2 Granulocyte colony-stimulating factor

The current treatment for immunological consequences is based on subcutaneous injections of G-CSF, with a starting dose of 1 μg/kg daily or alternate days, in case of severe persistent neutropenia and life threatening infection or documented IBD or severe diarrhea requiring hospitalization or disrupting normal life (10). The G-CSF dose should be gradually increased to obtain a neutrophil count of $0.5\text{-}1.0\times10^9$/l (3). However, G-CSF can improve neutrophil count but is ineffective on neutrophil dysfunction (6). Furthermore, it may worse splenomegaly and osteopenia (11) and increase the risk of malignancies (acute myeloid leukemia and myelodysplasia) (11–13). Evidences about the efficacy of G-CSF in treating GSDIb patients resulted from retrospectives studies and case reports (3).

3 Pathophysiology

The accumulation of 1,5-anhydroglucitol-6-phosphate (1,5-AG6P) within granulocytes was recently recognized as the cause of neutrophil dysfunction in GSDIb (14). The formation of 1,5-AG6P results from the action of hexokinases on 1,5-anhydroglucitol (1,5-AG, 1-deoxyglucose). This polyol, physiologically present in the blood, is a structural dietary analogue of glucose, which originates from dietary sources and has a constant blood concentration maintained by a renal clearance (15). However, it can also derive from an endogenous intestinal production (14). From neutrophil cytosol, G6PT transports 1,5-AG6P to endoplasmic reticulum, where is reconverted into 1,5-AG by glucose-6-phosphatase catalytic subunit 3 (G6PC3), homologous to G6PC1. In individuals lacking either G6PT (causing GSDIb) or G6PC3 (causing neutropenia type IV) 1,5-AG6P accumulates in neutrophils cytosol, where it inhibits the conversion of glucose into G6P by hexokinases. The reduced G6P lowers the glucose amount for glycolysis and pentose-phosphate pathway. In turn, the decreased ATP production from glycolysis affects neutrophil survival, and the defective NADPH production from the pentose-phosphate pathway impairs the respiratory burst. Also, the reduced UDP-glucose availability interferes with glycan formation and protein glycosylation (14) (Figure 1).

3.1 In vitro studies

Veiga da Cunha et al. used CRISPR-Cas9 to generate human HAP1 cell lines deficient in G6PC3 or G6PT, to investigate whether their deficiency leads to the accumulation of 1,5-AG6P. Addition of 1,5-AG to the culture media led to a dose-dependent accumulation of 1,5-AG6P in G6PC3- or G6PT-deficient cell lines, while its levels in wild-type cells remained ~100 times less. This demonstrated that 1,5-AG is phosphorylated into 1,5-AG6P and confirmed the hypothesis that both G6PC3 and G6PT are essential for the hydrolysis of 1,5-AG6P (14). Furthermore, consistent with the idea that 1,5-AG6P might inhibit the first step of glycolysis by inhibiting hexokinase 1 (as previously showed in brain (16)), the glycolytic intermediates (glucose-6-P, fructose-6-P, ribose-5-P, 6-phosphogluconate and fructose-1,6-bisphosphate) resulted significantly reduced.

Toxicity of 1,5-AG was first demonstrated in immortalized neutrophil progenitor cell line from G6PC3-deficient mice, in whom physiological concentrations of 1,5-AG in the culture medium caused massive accumulation of 1,5-AG6P, reduced G6P concentration, reduced glycolysis, reduced survival and cellular death (14).

3.2 In vivo studies

Leukocytes from 3 neutropenic patients with deficiency of G6PC3 or G6PT showed a concentration of 1,5-AG6P 500 times higher than controls. Furthermore, the concentration of 1,5-AG6P was higher in neutrophils than peripheral blood mononuclear cells in the same patient. These results were consistent with the idea that G6PT and G6PC3 are involved in 1,5-AG6P metabolism in human neutrophils and, when they are defective, 1,5-AG6P accumulates in the cytosol and inhibits hexokinases. Therefore, the consequent neutropenia is a metabolite-repair deficiency, caused by a failing to remove the nonclassical metabolite 1,5-AG6P (14).

4 Empagliflozin

Empagliflozin, an inhibitor of the renal sodium-glucose cotransporter type 2 (SGLT2), is an antidiabetic drug that inhibits

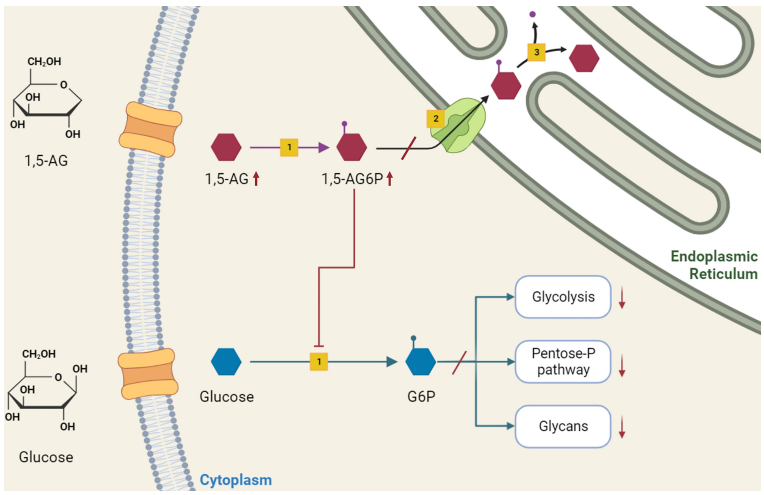

FIGURE 1
Role of 1,5-anhydroglucitol-6-phosphate accumulation in neutropenia and neutrophil dysfunction of G6PT deficiency. 1,5-AG, normally present in blood, enters into neutrophils where is slowly phosphorylated by a side activity of hexokinases [1] into 1,5-AG6P. G6PT [2] transports 1,5-AG6P into the endoplasmic reticulum, where it is dephosphorylated by G6PC3 [3]. In GSDIb patients, the deficiency of G6PT leads to 1,5-AG6P accumulation, which in turn inhibits hexokinase activity. This inhibition affects the conversion of glucose into G6P, interfering with downstream metabolic pathways of glycolysis, pentose-phosphate pathway and glycosylation, with consequent impaired neutrophil survival, respiratory burst and protein glycosylation.

the renal glucose reabsorption, causing the excretion of glucose and other sugars in diabetic and normoglycemic patients. 1,5-AG is normally filtered in the glomeruli and reabsorbed in the proximal renal tubules by specific active transporters (17). Since the reabsorption of 1,5-AG is competitively inhibited by glucose (18), conditions of increased glucosuria (such as uncontrolled diabetes or the use of SGLT2 inhibitors) lead to increase the urinary 1,5-AG excretion and lower its blood concentrations (6, 19–22).

Veiga da Cuhna et al. demonstrated that treatment with empagliflozin in G6PC3 deficient mice aimed to lower their blood level of 1,5-AG, decreased intracellular levels of 1,5-AG6P and normalized the neutrophil count. Conversely, treatment with 1,5-AG increased levels of 1,5-AG6P and provoked a neutrophil maturation arrest with accumulation of apparent promyelocytes (14).

4.1 Evidences from glycogen storage disease type Ib

After these results, the authors administered empagliflozin as off-label use in 4 GSDIb patients (3 children and 1 adult) with incomplete response to G-CSF to treat neutropenia and neutrophil dysfunction (6). Repurposing of empagliflozin therapy at the dosage of 0.3-0.7 mg/kg/day determined a decreasing of serum 1,5-AG and neutrophil 1,5-AG6P levels within one month, and patients showed clinical improvements with decreased symptoms of recurrent infections, mucosal lesions, and resolution of IBD. No symptomatic hypoglycemia was observed during treatment. G-CSF was discontinued in two patients and could be tapered by 81% and 57% in the remaining two patients, respectively. Neutrophil count increased and stabilized in all patients. Normalization of neutrophil oxidative burst, protein glycosylation, chemotaxis and bactericidal activity indicated the correction of neutrophil dysfunction (6).

G-CSF discontinuation after empagliflozin therapy was later reported in a 35-year old GSDIb patient with a concomitant improvement of wound healing and symptoms of IBD, without side effects (23).

Rossi et al. treated with empagliflozin a 14-year old GSDIb patient with severe IBD: he presented an improvement of clinical symptoms and stool frequency within the first week of therapy and normalization of the Pediatric Crohn disease activity index (PCDAI) within the first month. A significant decrease in disease activity was confirmed at the abdomen magnetic resonance imaging after 3 months of treatment, data confirmed on histology at 5.5 months. G-CSF dosage was reduced. Ameliorated metabolic control was noted in the present case. Stable lactate concentrations, normalization of triglycerides (TG) and recovery from hyperuricemia were reported. Liver and kidney function were normal and no adverse event was described. Healing from IBD might have yielded a positive effect on metabolic control, increasing intestinal glucose absorption and affecting the gut microbiota (24, 25), and certainly improved the overall psychosocial well-being of patient (24).

Another report confirmed that empagliflozin ameliorated neutropenia, neutrophil dysfunction, and IBD symptoms in a child, reducing serum 1,5-AG, with unchanged occasional hypoglycemia (26). Similar results were described in a 35-year old patient, who could reduce G-CSF dosage. No hypoglycemic event was reported (18).

Kaczor and coll. reported a series of 4 children treated with empagliflozin, confirming its positive effect on decreasing infections, improving IBD and wound healing. G-CSF was withdrawn or reduced. One patient experienced a single hypoglycemic episode during treatment, related to a delay of the nocturnal meal intake (27).

Grunert and coll. reported the favorable use of empagliflozin in a pregnant GSDIb patient: since the glycemic values were very unstable in the first weeks of gestation, the medication was reduced, causing a recrudescence of oral mucosal lesions, thereby the previous dose was restored. After the caesarean section, breastfeeding followed regularly. However, even the other reported patient, who was not treated with empagliflozin until childbirth, experienced recurrent mild hypoglycemia requiring glucose supply during pregnancy. G-CSF was discontinued in both patients, with normalization of neutrophil count and wound healing (28).

A group of 8 treated GSDIb pediatric patients with a median age of 7.8 years (range 1.5-15.8) was recently reported, with a cumulative treatment time more than 12 years: beneficial effects were showed on neutrophil function, anemia and growth. Plasma 1,5-AG levels reduced by a median of 78%. Half of the patients presented hypoglycemic episodes following the start of treatment, and one patient had hypoglycemic seizures: by lowering empagliflozin dosage and increasing carbohydrate intake, hypoglycemia resolved. Patients manifested an improvement in median Z-score for height and BMI, and a stable metabolic control (29).

Bidiuk et al. described 2 GSDIb adult siblings: in one suffering from heart failure, the treatment was associated to an improvement of cardiomyopathy, allowing the reduction of cardiological therapy and the withdrawn of eplerenone. Symptomatic hypoglycemia was not reported. The treatment with empagliflozin allowed to reduce the G-CSF dosage and the number of medications for concomitant clinical conditions. In his brother, who also suffered from HLA-B27 positive arthritis and sinus tachycardia, empagliflozin allowed corticosteroid withdrawal and antiarrhythmic therapy was tapered, without alterations in glycemic control (30).

Hexner-Erlichman and coll. reported 2 patients (17-year and 8-year old) who received empagliflozin for 18 months and 24 months, respectively. The treatment was safe and effective, resulting in normalization of neutrophil count and function, and yielded the resolution of mucosal lesions and improvement of IBD with discontinuation of G-CSF. No hypoglycemic event was recorded in both patients (31).

Using untargeted metabolomics profiling, Tallis et al. monitored empagliflozin treatment in an 11-year old girl, following the reduction of 1,5-AG levels; concurrently, neutrophil counts and function improved, as well as her IBD symptoms with reduction of G-CSF dose. Empagliflozin dose was adjusted because of arthralgia, but no other side effects were reported. Fasting tolerance improved (32). Untargeted metabolomics also showed

modifications of other metabolic pathways of lipids metabolism, as previously reported in GSDIb subjects (33), and empagliflozin-related improvement in other parameters of metabolic control, such as urate and urate intermediates (32).

Empagliflozin effects on neutrophil dysfunction, glycemic and metabolic control from the above-described case reports were detailed in Table 1.

Furthermore, the results of a multicenter study on the safety and efficacy of empagliflozin in GSDIb patients was recently reported. Clinical data from 112 subjects were collected through an international retrospective questionnaire from 24 countries. Empagliflozin was started at a mean age of 12.8 years (range 0-38), majority <18 years. Mean duration of treatment was 10.1 months (range <1-27), and the median dose was 0.35 mg/kg/day (range 0.1-0.9). Patients showed an improvement of mucosal lesions, recurrent infections and symptoms of IBD. The 5% discontinued G-CSF and 17% tapered the dosage. Hypoglycemia was reported in 18% of patients and was not related to the dose frequency. Lactic acidosis was reported in 5% of patients (5 children, 1 adult), requiring hospitalization. One of the adult individuals also had two hospitalizations in intensive care unit for ketoacidosis, during gastroenteritis and dehydration (34).

4.2 Evidences from neutropenia type 4

The first evidence for the use of empagliflozin in G6PC3 deficiency were recently described in 3 patients: a 30-year old woman with neutropenia, recurrent infections and inflammatory enterocolitis (35) and 2 children with recurrent infections (17). Improvement of neutropenia and colitis manifestations as well as of the quality of life was reported. In these patients, the dosage of empagliflozin (0.03-0.28 mg/kg/day) able to decrease plasma 1,5-AG was lower than GSDIb patients: a possible explanation could be that patients with G6PC3 deficiency do not take CS, an important source of 1,5-AG (36), therefore they may need a lower dose of empagliflozin to correct neutrophil dysfunction (35). Furthermore, mutation in other sodium-glucose transporters, such as SGLT5, might explain higher urinary excretion of 1,5-AG, milder neutropenia and a better responsiveness to empagliflozin in some patients (17).

4.3 Evidences from diabetes

4.3.1 Metabolic effects

A prospective trial (NCT01248364) was conducted to evaluate the metabolic effects of empagliflozin after a single dose and after four weeks of treatment in patients with type 2 diabetes (T2DM). Postprandial glucose and insulin AUC significantly decreased, whereas the glucagon increased. Fasting endogenous glucose production increased, and tissue glucose disposal was reduced. Chronic dosing shifted the substrate utilization from carbohydrates to lipids (37). Subsequent studies confirmed that the glucosuric effects of empagliflozin resulted in a decrease of plasma glucose levels (38–41), glycemic spikes and HbA1c levels (42). However, empagliflozin has pleiotropic effects, such as reducing insulin levels, insulin resistance, glucotoxicity and lipotoxicity, and increasing glucagon secretion and ketone bodies production (42, 43). Furthermore, empagliflozin improves redox state and oxidative stress, inhibiting reactive oxygen species (ROS) production, reducing the activity of pro-oxidant agents, and improving mitochondrial function (44). Of note, all these processes are implicated in the pathogenesis of metabolic, cardiovascular (CV), renal and neurological consequences of diabetes (42, 45). Particularly, in diabetes and obesity the excessive circulating free fatty acids accumulate in the adipose tissue mainly as TG. When fat storage exceeds subcutaneous and visceral adipose tissue capacity, ectopic fat accumulates in other tissues, such as skeletal muscle and myocardium, liver and pancreatic β-cells, with progressive impairment of mitochondrial function. This process of lipotoxicity generates a chronic low-grade inflammation and an insulin resistance state, which lead to impaired glucose tolerance, dyslipidemia, and hypertension (46, 47). Furthermore, the non-fasting hyperlipidemia causes endothelial stress through the production of inflammatory cytokines and oxidative stress agents, and the reduction of endothelial nitric oxide synthase activity (48, 49). Empagliflozin exerts protective effects from lipotoxicity, decreasing lipid accumulation in visceral fat (43) and promoting weight loss (50), reducing inflammation and oxidative stress (42).

Uric acid is another well-established pro-inflammatory mediator (51). Empagliflozin has been demonstrated to lower uric acid in T2DM patients with a likely positive effect on low-grade inflammation (50, 52). Several mechanism have been hypothesized, including the inhibition of renal tubular uric acid transporters in response to glucosuria and the inhibition of xanthine oxidase for uric acid production (53).

Conversely, the action of insulin on low-grade inflammation is more discussed, with different reports suggesting a time and dose-dependent activity (54, 55). However, in a recent study, patients on therapy with SGLT2-inhibitors (SGLT2-I) showed decreased levels of insulin and uric acid, and lower levels of IL-6, a marker of low-grade inflammation associated to diabetes complications (52).

4.3.2 Cardiac effects

Hyperglycemia along with inflammation and oxidative stress are the main cause of vascular dysfunction and CV disease in diabetes. The efficacy of empagliflozin in reducing CV risk and macrovascular complications in the diabetic population have been demonstrated in several pioneering clinical trials, such as EMPA-REG OUTCOME, EMPEROR-Reduced and EMPEROR-Preserved (56–58). Its diuretic and hypotensive effects are hemodynamic mechanisms (43) which involve several pathways. Glucosuria leads to a negative energy balance, which generates a 'fasting-mimicry' condition. Although the amount of SGLT2-I-induced urinary loss of glucose may result arduous to estimate, evidences from a caloric deficit come from patients with familial renal glucosuria type 1 (*SLC5A2* deficiency), in whom glucosuria can range up to 150 g/1.73 mq/day (59), which corresponds to ~600 kcal/day. The negative energy balance yields activation of the SIRT1/AMPK signaling pathway, with consequent suppression of Akt/mTOR signaling (60) and positive effect on metabolism, mitochondrial function and oxidative stress.

TABLE 1 Empagliflozin effects in GSDIb patients reports.

Study	Gender Age	Empagliflozin final dose	Treatment duration	1,5-AG§	Neutrophil count/ function	Skin-mucosal lesions	IBD	Hypoglycemic events	Lactate	Uric acid	TG	Micro-albu-minuria	Other treatments	Notes
Wortmann et al., 2020 (6)	♀ 21y	0.4 mg/kg/d (10 mg x 2/d)	288 d	↓	↑/↑	↓	↓	1 mild (2.7 mml/L) 9h after 1st dose, no other	na	na	na	na		Increased appetite and oral feeds
	♀ 2y	0.3 mg/kg/d (5 mg/d)	217 d	↓	↑/↑	↓	–	none	na	na	na	na		Increased appetite and oral feeds
	♂ 6y	0.7 mg/kg/d (10 mg x 2/d)	246 d	↓	↔/↑ ↓G-CSF	↓	↓	none	na	na	na	na	Sulfasalazine; antiepileptics; G-CSF (↓)	From drip feeding to oral feeds portions; improvement of glycemic control, no more hypoglycemia induced seizures
	♀ 2y	0.5 mg/kg/d (7.5 mg/d)	191 d	↓	↑/↑	↓	↓	Occasional (2,8 – 3.9 mmol/L) asymptomatic, reduced frequency	na	na	na	na	Sulfasalazine (stop)	↑ interval between meals; improvement of glycemic control
Grunert et al., 2020 (23)	♀ 34y	0.4 mg/kg/d (10 mg x 2/d)	>80 d	na	↔/↑ Stop G-CSF	↓	↓	none	na	na	na	–	G-CSF (stop); pancreatin, budesonide; vitamin E; loperamide (stop); 5-aminosalicylic acid	Stool frequency decreased; CDAI 398➔184
Mikami et al., 2021 (26)	♀ 30m	0.5 mg/kg/d	>60 d	↓	↑/↑	–	↓	Hypoglycemia (<2.8 mmol/L) before breakfast, frequency not changed by therapy	na	↓	↓	na	na	
Rossi et al., 2021 (24)	♂ 14y	0.4 mg/kg/d (10 mg x 2/d)	232 d	↓	↑/na ↓ G-CSF	–	↓	1st week: < 3.3 mmol/L on 20/32 low-glucose events at FGM, asymptomatic; 2–4 asymptomatic mild events/ month (2.7–3.3 mmol/L)	↔, in range	↑, in range*	↑, in range	–	G-CSF (↓); allopurinol (stop)	Juvenile idiopathic arthritis. From exclusive tube feeding, restart oral feeds portions (d164)

(Continued)

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TABLE 1 Continued

Study	Gender Age	Empagliflozin final dose	Treatment duration	1,5-AG§	Neutrophil count/ function	Skin-mucosal lesions	IBD	Hypoglycemic events	Lactate	Uric acid	TG	Micro-albu-minuria	Other treatments	Notes
Kaczor et al., 2022 (27)	♂ 17.5y	0.3 mg/kg/d	1.5 y	na	↑/na Stop G-CSF	↓	↓	none	↑, in range	↓, in range	↔, in range	–	G-CSF (stop); zoledronic; mesalazine	Stool frequency decreased; reduced hospitalization
	♂ 13y	0.4 mg/kg/d	1 y	na	↑/na Stop G-CSF	↓	↓	none	↓	↓, in range*	↓*	–	G-CSF (↓); mesalazine; allopurinol; fenofibrate; febuxostat	Stool frequency decreased; reduced hospitalization
	♀ 9y	0.4 mg/kg/d	1 y	na	↔/na ↓ G-CSF	↓	↓	One episode (1.44 mmol/L) related to delayed night meal	↔, in range	↓, in range	↔, in range	–	G-CSF (stop); mesalazine	Stool frequency decreased; reduced hospitalization
	♀ 17m	0.4 mg/kg/d	6 m	na	↑/na Stop G-CSF	↓		none	↑	↓, in range	↔, high	–	G-CSF (stop)	
Grunert et al., 2022 (28)	♀ 34y	na		na	↑/na	↓	–	na	na	na	na	–	G-CSF (stop); vitamins; L-thyroxine	Pregnancy, Empagliflozin started after delivery
	♀ 35y	2 x 10 mg/d		na	↑/na	↓	↔	Severe hypoglycemia (≈ 2.2 mmol/L) three times throughout pregnancy	na	na	na	–	G-CSF (stop); Phenprocoumon➔ enoxaparin	Empagliflozin started during pregnancy; breastfeeding
Tallis et al., 2022 (32)	♀ 11y	0.6 mg/kg/d (10 mg x 2/d)	22w		↔/↑ ↓ G-CSF	na	↓	After attempts to space fasting intervals	↑, in range	↓	na	–	G-CSF (↓)	Generalized arthralgia after dose escalation; PCDAI 35➔27.5; increase fasting time
Halligan et al., 2022 (29)	7y	0.2 mg/kg/d	1.7y	↓	↓/na Stop G-CSF	na	↔	4/8 patients experienced hypoglycemia upon start of treatment; 1/8 significant symptomatic hypoglycemic events including an overnight	na	↓	↓	na	2/8 allopurinol (1 stop, 1 ↓)	3♂,5♀; 3 of 4 pts studied had baseline neutrophil dysfunction, normalized after therapy Anemia resolved in all patients; PUCAI score
	15.2y	0.1 mg/kg htwt/d	1.3y	↓	↓/na	na	↓		na	↓	↔	na		
	8.6y	1.3 mg/kg htwt/d	1.83y	↓	↑/na Stop G-CSF	na	↓		na	↔	↑	na		
	4.3y	0.4 mg/kg htwt/d	2.3y	↓	↑/na Stop G-CSF	na	↓		na	↓	↑	na		

(Continued)

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TABLE 1 Continued

Study	Gender Age	Empagliflozin final dose	Treatment duration	1,5-AG[§]	Neutrophil count/ function	Skin-mucosal lesions	IBD	Hypoglycemic events	Lactate	Uric acid	TG	Micro-albu-minuria	Other treatments	Notes
	15.4y	0.2 mg/kg htwt/d	0.8y	↓	na/na	na	na	hypoglycemic seizure	na	na	na	na		improve in 4/6 patients. One patient had loss of previously established metabolic control, (elevated urate, triglycerides, and pre-feed lactate levels)
	15.8y	0.1 mg/kg htwt/d	1.8y	↓	↑/na	na	↓		na	↓	↔	na		
	1.5y	0.2 mg/kg htwt/d	0.6y	↔	↔/na	na	na		na	↓	↓	na		
	6.8y	0.2 mg/kg htwt/d	2.1y	↓	↔/na	na	↑		na	↓	↑	na		
Bidiuk et al., 2022 (30)	♂ 29y	20 mg/d	12m	na	↑/na ↓ G-CSF	↓	↓	none	↔, high	↔, high	↑ *	+	G-CSF (↓); metoprolol (↓); ramipril (↓); eplerenone (stop); fenofibrate (start) [other therapies, not specified]	Heart failure; reduction of the number of medications
	♂ 28y	20 mg/d	12m	na	↑/na ↓ G-CSF	–	↔	none	↔, high	↔, high	na	+	G-CSF (↓); metoprolol (↓); ivabradine (↓); methylprednisolone (stop); allopurinol; sulfasalazine (↓); fenofibrate [other therapies, not specified]	HLA-B27 positive arthritis; wheel-chair-bound; sinus tachycardia; reduction of the number of medications
Makrilakis et al., 2022 (18)	♀ 32y	0.4 mg/kg/d (25 mg/d)	>300 d	↓	↑/na	na	↓	none	na	↔, in range	↔, in range	na	G-CSF (↓); allopurinol; mesalazine (stop); denosumab	CDAI 356➔52
Hexner-Erlichman et al, 2022 (31)	♀ 17.5y	0.65 mg/kg/d	18m		↑/na	↓	↓	none	na	na	na	+	G-CSF (stop); ACEi; prednisone (stop); sulfasalazine; fibrate; omega-3 fatty acids	Glomerular and tubular dysfunction; chronic pancreatitis
	♂ 8.5y	0.5 mg/kg/d	24m		↑/na	↓		none	na	na	na	–	Endocrine and exocrine pancreatic replacement therapy	

[§]Reference values different for every work, see references.; *Concomitant therapy.

ACEi, ACE inhibitor; CDAI, Crohn's Disease Activity Index; mg/kg htwt, milligrams per kilogram of height weight; na, not available; PCDAI, Pediatric Crohn's Disease Activity Index; PUCAI, Pediatric Ulcerative Colitis Activity Index; TG, triglycerides; ↑, increase; ↓, decrease; ↔, stable; +, pre-existing, no data on empagliflozin; -, absent.

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Oxidative stress from dysfunctional mitochondria plays a pivotal role in diabetic cardiomyopathy. Remarkably, in diabetes the increased free fatty acids accumulate in cardiomyocytes as lipid droplets containing TG, diacylglycerol (DAG) and ceramides which contribute to the development of left ventricular hypertrophy and cardiac dysfunction (lipotoxicity) (61), and to the exacerbation of insulin resistance (43). The latter reduces the glucose utilization and overbalances the lipidic metabolism towards fatty acids accumulation, which leads to progressive mitochondrial dysfunction and consequent increased oxidative stress. This process in turn aggravates the diabetic cardiomyopathy and myocardial dysfunction (62).

Empagliflozin has been proved to activate AMPK in mice cardiomyocytes and restore energy levels (63), via acetyl coenzyme A carboxylase (ACC) inhibition, which enhances fatty acid oxidation and increases ATP generation (64). Furthermore, AMPK activation plays a main role in stimulating autophagy and in turn having cardio- and mitochondrial protective effects (60, 65) (Figure 2). Of note, mitochondrial autophagy (mitophagy) which is regulated by several signaling pathways, including AMPK-mTOR (43), plays a cardioprotective role through the clearance of abnormal mitochondria, thus preventing the oxidative stress and reducing the myocardiocytes apoptosis (66).

Furthermore, empagliflozin down-regulates the Na^+/H^+ antiport, reducing the cytoplasmic sodium and calcium levels and increasing the mitochondrial calcium concentrations, which in turn enhances the ATP synthesis and improves the cardiac contractile activity (67), regardless the antihyperglycemic effect (68).

In addition, studies conducted on mice and rats models suggested protective effects of SGLT2-I against the development of fibrosis in different types of cardiopathy and nephropathy (69–71).

4.3.3 Renal effects

The SGLT2-I drugs reduce the reabsorption of glucose and sodium, leading to a diuretic osmotic effect. Differently from loop diuretics, SGLT2-I causes a greater reduction of fluids in the interstitial compartment, resulting in a lower depletion of circulating volume and minor effect on tissue perfusion (72). In type 1 diabetes patients with hyperfiltration (GFR > 135 ml/min), empagliflozin has been documented to reduce the intraglomerular pressure and the glomerular filtration rate (GFR) of – 30 ml/min, through the sodium reabsorption (73). Also T2DM patients are at risk of developing diabetic nephropathy. Hyperfiltration is the first stage of nephropathy, which may progress towards glomerular fibrosis, renal failure and end-stage renal disease (74). Several trials showed that empagliflozin yielded significant renoprotective effects in subjects with T2DM (75–79) and end-stage kidney disease (80). Furthermore, it lowered the estimated GFR and reduced the urine albumin-to-creatinine ratio in both microalbuminuric and macroalbuminuric ranges (81, 82). Therefore, the 2021 ADA guidelines recommended SGLT2-I in T2DM patients with nephropathy to lower the risk of renal failure, major CV events and heart failure hospitalization (83).

Inhibition of SIRT1/AMPK signaling and autophagy is also involved in the pathogenesis of the glomerular and tubular lesions in diabetic nephropathy, particularly in the tubular accumulation of advanced glycation end products and in the podocyte injury (84–86). SGLT2 expression is stimulated by glucose, acting as a sensor of energy abundance. Its activity is inversely related to SIRT1

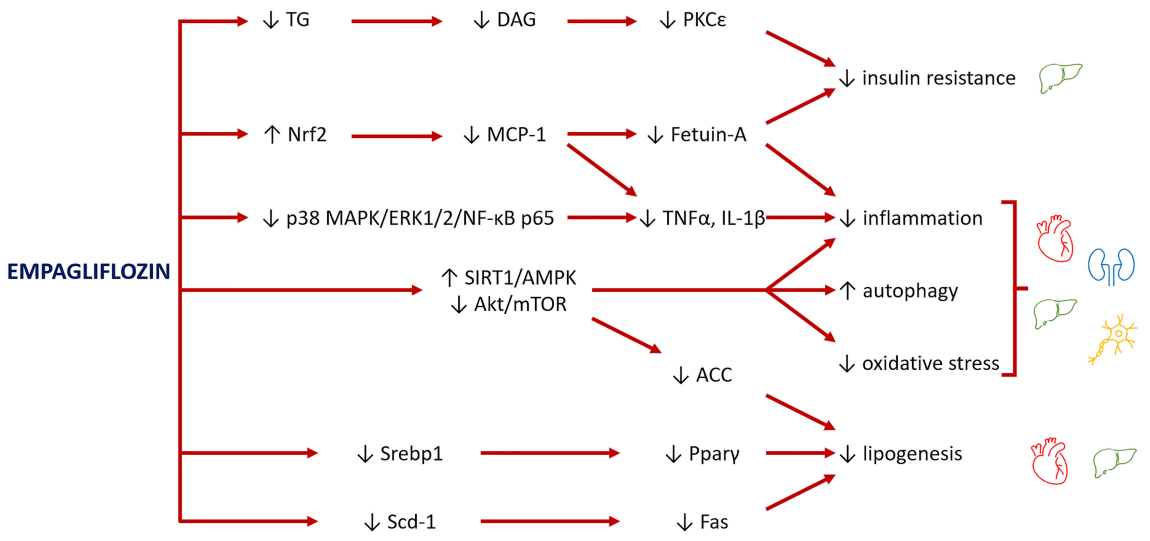

FIGURE 2
Major pathways involved in the rescue of organ damage from empagliflozin. Empagliflozin reduces insulin resistance by decreasing the levels of triglycerides (TG) and their lipotoxic intermediates diacylglycerols (DAG), lowering the inhibitory effect of PKCε on insulin receptor. Empagliflozin also increases levels of Nrf2, a mediator between lipid metabolism and antioxidant defense, with consequent inhibition of MCP-1 and fetuin-A and reduction of inflammation and oxidative stress. A further mechanism to inhibit inflammation is through suppression of p38 MAPK/ERK1/2/NF-κB p65 expression. The main role of empagliflozin is activation of SIRT1/AMPK and inhibition of Akt/mTOR pathways, which reduce inflammation, improve mitochondrial function and autophagy, and decrease oxidative stress. Finally, empagliflozin reduces the levels of transcription factors (Srebp1 and Pparγ) and the expression of enzymes (Scd-1, Fas), leading to a reduction of lipogenesis, with benefits especially for the heart and liver.

expression and autophagic activation in the kidney (87). Therefore, pharmacological modulation of SGLT2 may have a main role in modifying the energy/redox sensing molecules to stimulate autophagy (60) (Figure 2). Remarkably, there are evidences that empagliflozin ameliorated diabetic tubulopathy and renal fibrosis by controlling autophagy in mice (88).

4.3.4 Hepatic effects

Non-alcoholic fatty liver disease (NAFLD) is a wide spectrum of chronic liver disorders, ranging from steatosis to non-alcoholic steatohepatitis, fibrosis, and cirrhosis (89). The association between NAFLD and T2DM increases the risk of advanced liver damage (90). Furthermore, T2DM patients with NAFLD are at higher CV risk, suggesting that these conditions share common pathogenic mechanisms, such as low-grade systemic inflammation and oxidative stress (91).

In a prediabetic rat model, empagliflozin had ameliorative effects on hepatic lipid metabolism regardless obesity, by decreasing lipogenesis, fetuin-A and inflammation (92, 93) (Figure 2). Fetuin-A is an hepatokine which represents an important link between adipose tissue, liver and skeletal muscle (92). It promotes insulin resistance by a direct action on the insulin receptor (93) and stimulates the low-grade inflammation binding the toll-like receptor 4, thus producing a lipid-induced pro-inflammatory response (94). By decreasing serum concentration of fetuin-A, empagliflozin likely exerted an ameliorative effect on lipid metabolism, insulin resistance and inflammation in liver as well as in peripheral tissues (95).

In the liver, empagliflozin lowered both hepatic neutral TG and lipotoxic DAG (92, 96–98). These compounds are capable to induce insulin resistance and endoplasmic reticulum stress (99). DAG can activate PKCε, which inhibits the insulin receptor by its phosphorylation (98), inducing insulin resistance and endoplasmic reticulum stress (99), both involved in hepatic steatosis development. Empagliflozin administration in the pre-diabetic rats improved hepatic and peripheral insulin resistance mainly decreasing hepatic DAG, but also significantly reducing plasma insulin levels. As a consequence, the rats showed lower blood glucose levels and improved glucose tolerance, decreased fasting glucose, postprandial glucose and AUC, and increased insulin sensitivity in skeletal muscle (95).

In a study conducted on diabetic patients, empagliflozin yielded a reduction of liver fat content (100). Another study investigated the effects of SGLT2-I on markers of oxidative stress, inflammation, liver steatosis and fibrosis in subjects with uncontrolled T2DM and NAFLD. Twenty-six patients treated with metformin were introduced to a SGLT2-I or other glucose-lowering drugs (OTHER) (101). After six months of treatment, a reduction of fatty liver index and the FibroScan result was displayed in the SGLT2-I group. In addition, reduced serum levels of IL-1β, IL-6, TNF, VEGF and MCP-1, and higher levels of IL-4 and IL-10 were reported in the SGLT2-I group. Serum HNE- or MDA-protein adducts (systemic markers of oxidative stress caused by lipid peroxidation) decreased significantly in SGLT2-I patients and correlated with liver steatosis and fibrosis scores. SGLT2-I therapy was associated with reduced liver steatosis and fibrosis markers, circulating pro-inflammatory cytokines and oxidative biomarkers, regardless the glycemic control (101).

4.3.5 Neuroprotective effects

Multiple pathogenic mechanisms have been involved in diabetic peripheral neuropathy (DPN), such as PKC, the polyol pathways, the formation of the advanced glycation end products, and oxidative stress (102). Subsequentially, nerve energy production is reduced (103, 104), accompanied by a disturbance in proteins axonal transport (105). Inhibition of the AMPK pathway hampered mitochondrial function and caused neuronal damage in diabetic rats (106). Conversely, activation of the AMPK expression prevented streptozotocin-induced neuroinflammation enhancing the mitochondrial biogenesis and autophagy in vivo (107), and stimulated the expression of antioxidant enzymes in vitro (108).

In diabetic rats, empagliflozin has been shown to improve sciatic nerve histopathological modifications, scoring, myelination, nerve fibers' count, and nerve conduction velocity, independently from its anti-hyperglycemic effect. Moreover, it ameliorated motor coordination and mitigated responses to nociceptive stimuli (44). Empagliflozin displayed a protective effect against DPN via regulating the AMPK signaling to reduce oxidative and inflammatory burden, to regulate the extracellular matrix remodeling, and to stimulate autophagy (45, 107). The authors hypothesized a restoration of energy levels, via AMPK activation, as in cardiomyocytes (63, 64). Furthermore, mTor expression resulted reduced by 47% after empagliflozin administration in the sciatic nerve (45). mTOR inhibition might improve DPN through activation of autophagy to remove any damaged cellular constituents, thus increasing the myelin thickness and the myelinated axons (109) and reducing pain (110). Moreover, empagliflozin ameliorated the deleterious effect of inflammatory mediators (IL-1β and TNF-α) by suppressing p-p38 MAPK/p-ERK1/2/p-NF-κB p65 expression (45) (Figure 2).

5 Discussion

5.1 Empagliflozin use in glycogen storage disease type Ib

In GSD type I, the nutritional management is the mainstay of treatment, consisting of high carbohydrate diet, frequent feeding, nocturnal enteral feeding and CS. The aim of nutrition is to achieve a good glycemic and metabolic control and to prevent the long-term complications, such as liver adenoma, hepatocarcinoma, nephropathy and osteoporosis (111). However, patients with GSDIb often present difficulties with the management of nasogastric tube or gastrostomy for the risk of delayed wound healing or infection (6, 23, 26–28, 30, 31), and inflammatory bowel disease.

Data from case reports and a questionnaires from 112 patients showed that treatment with empagliflozin has significant beneficial

effects on neutrophil dysfunction, mucosal lesions, recurrent infections, anemia and bowel diseases (6, 18, 23, 24, 26–32). Furthermore, the majority of patients displayed an amelioration of glycemic control with a better fasting tolerance and metabolic parameters (6, 24, 27, 31). Also, increased appetite and recover from tube feeding-dependence has been reported, with an improvement of general well-being (6, 23, 24, 29, 32).

The SGLT2-I empagliflozin, registered for T2DM in adults, has a favorable safety profile. Current evidence from randomized controlled trials in T2DM does not indicate an increased risk of diabetic ketoacidosis and acute kidney injury for SGLT2-I (112). The most common adverse effects are increased urogenital fungal skin infections because of glucosuria. Hypoglycemia has rarely been observed only in association with other antidiabetic therapy (113).

Treating with a glucose-lowering drug patients with a metabolic disease presenting with hypoglycemia might sound counterintuitive, however low rates of hypoglycemia and other side effects, such as urogenital infections, lactic acidosis and ketoacidosis, have been reported so far in the first GSDIb patients treated with empagliflozin (6, 29, 34).

Benefits on glycemic control and fasting tolerance, beyond the favorable effects on neutrophil dysfunction, have been well described in the first study reporting the use of empagliflozin in GSDIb patients at the dose of 0.3-0.7 mg/kg/day (6). Particularly, glycemic profile was assessed by continuous glucose monitoring (CGM) and frequent capillary measurements for a maximum follow-up of 288 days. Mild asymptomatic hypoglycemia was occasionally registered, mostly in the first period of treatment and at a lower frequency than before treatment. Increased appetite was reported after three days in one patient. In another one, continuous enteral feeding was replaced by frequent meals and CS, hypoglycemic episodes quickly became less frequent, and CS was progressively reduced; after one month, the nasogastric tube was dismissed, and fasting intervals increased up three hours. The authors suggested that the improvement of IBD that affects intestinal nutrients absorption might have a positive impact on glycemic and metabolic control (6).

Another report from a GSDIb patient with bowel disease treated with G-CSF and continuous gastric deep feeding showed events of asymptomatic mild hypoglycemia < 3.3 mmol/L (range 2.6–3.3) measured by flash glucose monitoring during the first week of treatment and promptly corrected by oral glucose administration (24). Notably, accuracy of CGM devices in the hypoglycemia range is low and hypoglycemic events should be always verified by glucometer (114–116). However, time below range (TBR) decreased and time in range (TIR) increased also after oral refeeding. An interval of 2.5 h free-gastric drip feeding was introduced in the morning. Asymptomatic mild hypoglycemia was occasionally revealed (2–4 episodes/month) during the next six months by self-monitoring with glucometer (24).

Other case reports displayed an improved glycemic control (27, 31) or did not reveal any increased hypoglycemia (18, 26, 27, 30).

Conversely, in the Halligan paper (29), 4 out 8 patients experienced hypoglycemia after starting empagliflozin. These patients were managed by reducing empagliflozin dose and lightly increasing carbohydrates intake. However, patients were maintained in a tight dietary control in order to minimize carbohydrate intake.

Data from an international retrospective questionnaire study showed hypoglycemia in 20 out 112 patients (18%), lactic acidosis in 6 out 112 (5%). However, hypoglycemia and lactic acidosis are common findings in patients with GSDIb, therefore a contribute of empagliflozin treatment could not be determined. One patient required hospitalizations in intensive care unit for ketoacidosis, during gastroenteritis and dehydration. Therefore, the authors suggested eventually discontinuing empagliflozin during fever or gastrointestinal illnesses for a few days in GSDIb patients, until glucose and fluid homeostasis are stabilized (34). However, in conditions of genetic insulin resistance such as Rabson–Mendenhall syndrome under insulin therapy, empagliflozin improved glycemic control without significantly increasing ketonemia (117, 118).

In the context of metabolic alterations of GSDIb, patients can manifest hyperuricemia and hypertriglyceridemia. Hyperuricemia can result from the inhibitory effect on uric acid tubular excretion by lactate and an increased adenine nucleotides catabolism (119). Although only a few authors reported pre-post SGLT2-I values of uric acid and some cases were concurrently on allopurinol to prevent renal damage (120), empagliflozin seems to have ameliorative effects on hyperuricemia in some GSDIb patients (24, 26, 29, 32), being reported an improvement in the median urate levels (29), with discontinuation of allopurinol in two patients (24, 29). Similar data were evidenced by exploiting untargeted metabolomics profiling in one patient (32). If this may be related to a direct correlation between plasma uric acid and serum 1,5-AG levels, regardless glucosuria, is still unclear (121). Alternatively, this might be reflective of improved metabolic control (29).

TG values were reported to have a variable trend during therapy (26, 27, 29).

Since majority of the studies focused on the effect of empagliflozin on neutrophil dysfunction and inflammatory bowel disease and lack of a long-term follow-up, the current data were scant to evaluate the effect of empagliflozin on the liver, kidney and CV disease in GSDIb patients. However, in two patients with cardiac involvement (heart failure, hypertension and sinus tachycardia), on empagliflozin it was possible to taper the standard cardiological therapy without worsening signs and symptoms (30). This might be related to a possible beneficial effect of empagliflozin on heart function.

In summary, from the existing evidences glycemic and metabolic control appears to be improved in GSDIb patients under treatment with empagliflozin.

5.2 Perspectives of empagliflozin use in other metabolic diseases

Data about the systemic effects of empagliflozin have been gathered by trials conducted on big cohorts of T2DM patients to study CV and renal effects (56–58, 75–77), hepatic effects (122), and in preclinical studies (95, 123). Inducing a pseudo-fasting state, empagliflozin stimulates glycogen and lipid catabolism, and

ketogenesis, shifting the oxidative metabolism from carbohydrate to lipid utilization. Remarkably, during starvation, cells activate a transcriptional program of adaptation to shortage conditions. The pathways of SIRT1/AMPK and Akt/mTOR act as energy sensors. Empagliflozin can enhance SIRT1/AMPK and inhibit Akt/mTOR activity, leading to decrease lipogenesis, improve mitochondrial function and autophagy, and reduce oxidative stress and inflammation.

Several studies documented that empagliflozin activates the AMPK pathway in cardiomyocytes (65) and in a cardiac ischemia model in mice (64), hearts of lipopolysaccharide-treated mice (63), kidneys of diabetic mice (88), DPN of diabetic rats (45) as well as individual with T2DM (60) and in healthy conditions in vivo and in vitro (63).

Modulation of SIRT1/AMPK and Akt/mTOR pathways is crucial in reducing insulin levels, insulin resistance, glucotoxicity and lipotoxicity, and in increasing ketogenesis (60). At a cardiac level, this translates into reduction of coronary microvascular injury and cardiomyopathy and improvement of contractility (65). In the kidney, it ameliorates glomerular hyperfiltration, tubular damage and inflammation and mitigates the development of nephropathy (124–126). In the liver, empagliflozin protects from lipotoxicity and NAFLD through several mechanisms: reducing lipogenesis, modifying the expression of cytochrome proteins, increasing Nrf2, decreasing fetuin-A and reducing circulating pro-inflammatory cytokines (95). In the peripheral nerve, empagliflozin restores ATP production and enhances autophagy to remove any damaged cellular elements and meet the cellular energy needs (45).

Systemic effects of empagliflozin and its putative molecular mechanisms of action are depicted in Figure 3.

The above-mentioned pathways of cellular dysfunction are likely involved in the development of the organ damage in other metabolic diseases presenting with hypoglycemia and/or energy impairment, such as other types of GSDs (3, 127, 128) displaying hepatopathy with several degrees of NAFLD (GSDIa, III, IV, VI, IX, XI), cardiomyopathy (GSDIII), nephropathy (GSDIa); β-oxidation defects (129), mitochondrial disorders (130) with cardiomyopathy, nephropathy, hepatopathy and neuropathy. Furthermore, mitochondrial function and autophagy are defective in organic acidemias (131) and lysosomal storage diseases (132). Therefore, for its multiple effects empagliflozin might be repurposed in other inborn errors of metabolism.

5.2.1 Empagliflozin use in other types of glycogen storage diseases

GSDIa patients present with liver steatosis, because the accumulation of G6P in the endoplasmic reticulum activates lipogenesis (133) and is involved in mitochondrial stress and insulin resistance (134, 135). In GSDIa patients, increased plasma acylcarnitines and abnormal urinary organic acids profile suggested mitochondrial impairment. Remarkably, mitochondrial overload might generate intermediate molecules interfering with the insulin signaling and causing insulin resistance (136). However, insulin resistance and metabolic syndrome can also occur in case of overtreatment in GSDI (137, 138).

Furthermore, the pathogenesis of renal disease in GSDIa and Ib patients is similar to diabetic nephropathy, with initial

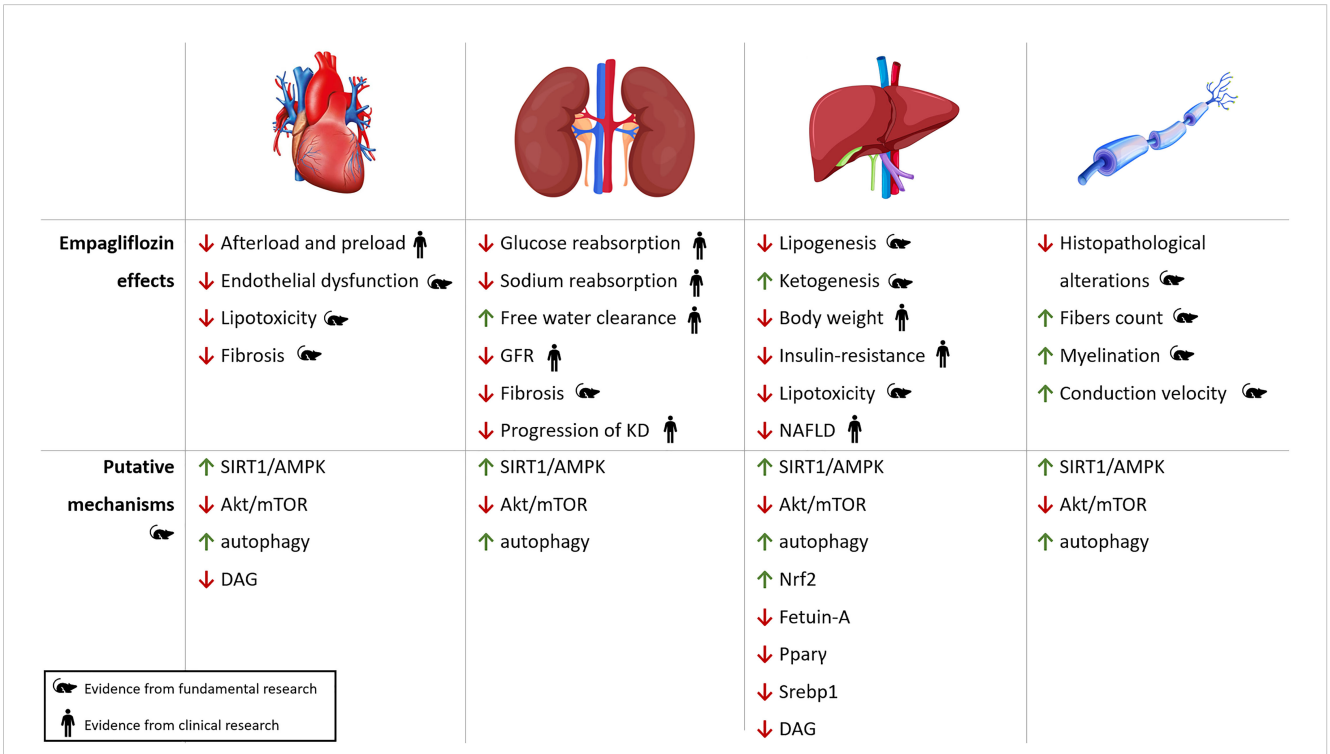

FIGURE 3
Pleiotropic effects of empagliflozin. Cardiac, renal, hepatic and neuropathic effects of empagliflozin with putative molecular pathways involved in oxidative stress, autophagy and inflammation. KD, kidney disease; NAFLD, Non-Alcoholic Fatty Liver Disease; ROS, reactive oxygen species.

hyperfiltration evolving towards microalbuminuria, proteinuria, glomerulosclerosis and renal failure (3). A stable renal function was mentioned in a single report of a GSDIb patient treated with empagliflozin (24). Whether empagliflozin use in GSDIb patients might have a protective effect on renal disease needs to be established in prospective trials.

Empagliflozin could be potentially used also in patients with GSDIa with the aim to improve metabolic parameters, mitochondrial stress, insulin resistance, NAFLD and nephropathy.

In GSDIII, the deficiency of the glycogen debranching enzyme results in the accumulation of an abnormal glycogen (limit-dextrin) in liver and muscles. Beyond hypoglycemia and hepatopathy, the cardiac glycogen storage leads to a hypertrophic cardiomyopathy, which can be obstructive and evolve towards heart failure. Standard dietary intervention with high protein and cornstarch supplementation has beneficial effect on glycemic control, but it can worsen glycogen accumulation in the heart. The only effective treatment for cardiomyopathy is ketogenic diet but the outcome strongly depends from patients compliance (127). Empagliflozin might be used to take advantage of its favorable effects on liver and heart.

Dysfunction of autophagy can lead to various liver diseases including NAFLD, steatosis, fibrosis, cirrhosis and hepatocellular carcinoma (139). Liver involvement is a constant finding in hepatic GSDs. Patients with GSDI easily develop steatosis and are at risk for liver adenoma and carcinoma (3, 133). Patients with GSDIII (140), IV, VI, IX, XI are at risk for progressive liver disease and tumor degeneration (127). Mechanisms of inhibition of autophagy are likely to be involved. Remarkably, impaired autophagy has recently been demonstrated in a mouse model of GSDIb (141). For its pro-autophagic effect, empagliflozin may represent a good candidate for the treatment of hepatic GSDs.

Some studies conducted on individuals with muscle GSDs type V and VII reported a beneficial effect of ketogenic diet on mitochondrial function, stimulation of β-oxidation and anaerobic glycolysis, and improvement of the work-out performance (142–145). Since the effect of empagliflozin on oxidative metabolism (shifted from carbohydrate to lipid oxidation) is similar to ketogenic diet, muscle GSDs could benefit of empagliflozin administration in the place or in conjunction with ketogenic diet.

5.2.2 Empagliflozin use in β-oxidation defects, mitochondrial disorders and organic acidemias

Patients with β-oxidation defects present with hypoglycemia, recurrent rhabdomyolysis, hepatopathy, cardiomyopathy and neuropathy. Oxidative disbalance and mitochondrial dysfunction are associated with these disorders (129). Along with patients with mitochondrial disorders and multiorgan involvement, they could benefit of antioxidant and protective effects of empagliflozin on mitochondrial function and mitophagy.

In organic acidemias the accumulation of toxic intermediate metabolism derivatives within mitochondria causes morphological and functional abnormalities leading to severe multiorgan dysfunction. Studies from urinary tubular cells, kidney tubules and explanted livers from methylmalonic acidemia (MMA) patients showed damaged and dysfunctional mitochondria, with generation of ROS and cell distress. A pathway connecting *MMUT* deficiency and mitophagy has recently been identified in mmut-deficient zebrafish. Restoring mitophagy ameliorated disease-relevant phenotypes in mmut-deficient zebrafish. These findings offer potential therapeutic perspectives for repairing mitochondria in MMA (131).

5.2.3 Empagliflozin use in lysosomal storage diseases

Finally, the role of defective autophagy in lysosomal storage diseases (LSDs) is well established. Absent or defective hydrolases or dysregulated endosomal-lysosomal processes lead to accumulation of macromolecules. For some LSDs the enzyme replacement therapy (ERT) is available with variable results. Autophagy inducers have already shown benefit in a few LSDs models (Niemann- Pick type B and C1, Gaucher, Pompe, Ceroid lipofuscinosis neuronal disease type 3). Combination strategies with empagliflozin to induce autophagy might prove more effective than ERT alone (132).

6 Conclusion

Although data come from case reports and a retrospective study, the recent off-label use of empagliflozin in GSDIb patients provided evidence of safety and efficacy in improving neutrophil dysfunction and related clinical phenotype. Majority of patients manifested also an improvement in glycemic and metabolic control with a favorable impact on general well-being. Further studies are waited for a more rigorous assessment of the long-term risks and benefits of therapy. Empagliflozin has pleiotropic systemic effects, for which it appears to be a good candidate for drug repurposing also in other metabolic diseases presenting with hypoglycemia, oxidative disbalance and mitochondrial dysfunction, inflammation and defective autophagy, with consequent organ damage.

Future directions through high quality studies should be aimed to provide evidences of safety and efficacy of the empagliflozin use in the inborn errors of metabolism, ranging over amino acids, carbohydrates and fatty acids metabolism defects, mitochondrial and lysosomal diseases.

Author contributions

AM conceptualized and designed the study, searched for literature, drafted the initial manuscript, and reviewed and approved the final manuscript as submitted. FT 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.

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.

The reviewer TD declared a past co-authorship with the author AM to the handling editor.

Publisher's note

References

1. Chou JY, Jun HS, Mansfield BC. Type I glycogen storage diseases: disorders of the glucose-6-phosphatase/glucose-6-phosphate transporter complexes. *J Inherit Metab Dis* (2015) 38:511–9. doi: 10.1007/s10545-014-9772-x

2. Veiga-da-Cunha M, Gerin I, Chen Y-T, de Barsy T, de Lonlay P, Dionisi-Vici C, et al. A gene on chromosome 11q23 coding for a putative glucose- 6-phosphate translocase is mutated in glycogen-storage disease types ib and ic. *Am J Hum Genet* (1998) 63:976–83. doi: 10.1086/302068

3. 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–e29. doi: 10.1038/gim.2014.128

4. Visser G, Rake J-P, Fernandes J, Labrune P, Leonard JV, Moses S, et al. Neutropenia, neutrophil dysfunction, and inflammatory bowel disease in glycogen storage disease type ib: results of the European study on glycogen storage disease type I. *J Pediatr* (2000) 137:187–91. doi: 10.1067/mpd.2000.105232

5. Chen MA, Weinstein DA. Glycogen storage diseases: diagnosis, treatment and outcome. *Transl Sci Rare Dis* (2016) 1:45–72. doi: 10.3233/TRD-160006

6. 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

7. Melis D, Parenti G, Della Casa R, Sibilio M, Berni Canani R, Terrin G, et al. Crohn's-like ileo-colitis in patients affected by glycogen storage disease ib: two years' follow-up of patients with a wide spectrum of gastrointestinal signs. *Acta Paediatr* (2003) 92:1415–21. doi: 10.1080/08035250310007033

8. Wicker C, Roda C, Perry A, Arnoux JB, Brassier A, Castelle M, et al. Infectious and digestive complications in glycogen storage disease type ib: study of a French cohort. *Mol Genet Metab Rep* (2020) 23:100581. doi: 10.1016/j.ymgmr.2020.100581

9. Rake JP, Visser G, Labrune P, Leonard JV, Ullrich K, Smit GPA. European Study on glycogen storage disease type I (ESGSD i). guidelines for management of glycogen storage disease type I - European study on glycogen storage disease type I (ESGSD I). *Eur J Pediatr* (2002) 161 Suppl:S112–9. doi: 10.1007/s00431-002-1016-7

10. Visser G, Rake J, Labrune P, Leonard J, Moses S, Ullrich K, et al. Consensus guidelines for management of glycogen storage disease type 1b - European study on glycogen storage disease type 1. *Eur J Pediatr* (2002) 161:S120–3. doi: 10.1007/s00431-002-1017-6

11. Visser G, Rake J, Labrune P, Leonard J, Moses S, Ullrich K, et al. Granulocyte colony-stimulating factor in glycogen storage disease type 1b. results of the European study on glycogen storage disease type 1. *Eur J Pediatr* (2002) 161:S83–7. doi: 10.1007/s00431-002-1010-0

12. Li AM, Thyagu S, Maze D, Schreiber R, Sirrs S, Stockler-Ipsiroglu S, et al. Prolonged granulocyte colony stimulating factor use in glycogen storage disease type 1b associated with acute myeloid leukemia and with shortened telomere length. *Pediatr Hematol Oncol* (2018) 35:45–51. doi: 10.1080/08880018.2018.1440675

13. Dale DC, Bolyard AA, Marrero T, Kelley ML, Makaryan V, Tran E, et al. Neutropenia in glycogen storage disease ib. *Curr Opin Hematol* (2019) 26:16–21. doi: 10.1097/MOH.0000000000000474

14. Veiga-da-Cunha M, Chevalier N, Stephenne X, Defour JP, Paczia N, Ferster A, et al. Failure to eliminate a phosphorylated glucose analog leads to neutropenia in patients with G6PT and G6PC3 deficiency. *Proc Natl Acad Sci USA* (2019) 116:1241–50. doi: 10.1073/pnas.1816143116

15. Yamanouchi T, Tachibana Y, Akanuma H, Minoda S, Shinohara T, Moromizato H, et al. Origin and disposal of 1,5-anhydroglucitol, a major polyol in the human body. *Am J Physiol Metab* (1992) 263:E268–73. doi: 10.1152/ajpendo.1992.263.2.E268

16. Crane RK, Sols A. The non-competitive inhibition of brain hexokinase by glucose-6-Phosphate and related compounds. *J Biol Chem* (1954) 210:597–606. doi: 10.1016/S0021-9258(18)65385-2

17. Boulanger C, Stephenne X, Diederich J, Mounkoro P, Chevalier N, Ferster A, et al. Successful use of empagliflozin to treat neutropenia in two G6PC3-deficient children: impact of a mutation in SGLT5. *J Inherit Metab Dis* (2022) 45:759–68. doi: 10.1002/jimd.12509

18. Makrilakis K, Barmpagianni A, Veiga-da-Cunha M. Repurposing of empagliflozin as a possible treatment for neutropenia and inflammatory bowel disease in glycogen storage disease type ib: a case report. *Cureus* (2022) 14:12–5. doi: 10.7759/cureus.27264

19. Akanuma Y, Morita M, Fukuzawa N, Yamanouchi T, Akanuma H. Urinary excretion of 1,5-anhydro-D-glucitol accompanying glucose excretion in diabetic patients. *Diabetologia* (1988) 31:831–5. doi: 10.1007/BF00277486

20. Chao EC, Henry RR. SGLT2 inhibition — a novel strategy for diabetes treatment. *Nat Rev Drug Discov* (2010) 9:551–9. doi: 10.1038/nrd3180

21. Fortuna D, McCloskey LJ, Stickle DF. Model analysis of effect of canagliflozin (Invokana), a sodium–glucose cotransporter 2 inhibitor, to alter plasma 1,5-anhydroglucitol. *Clin Chim Acta* (2016) 452:138–41. doi: 10.1016/j.cca.2015.11.010

22. DeFronzo RA, Hompesch M, Kasichayanula S, Liu X, Hong Y, Pfister M, et al. Characterization of renal glucose reabsorption in response to dapagliflozin in healthy subjects and subjects with type 2 diabetes. *Diabetes Care* (2013) 36:3169–76. doi: 10.2337/dc13-0387

23. Grünert SC, Elling R, Maag B, Wortmann SB, Derks TGJ, Hannibal L, et al. Improved inflammatory bowel disease, wound healing and normal oxidative burst under treatment with empagliflozin in glycogen storage disease type ib. *Orphanet J Rare Dis* (2020) 15:218. doi: 10.1186/s13023-020-01503-8

24. 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) 47:149. doi: 10.1186/s13052-021-01100-w

25. Ceccarani C, Bassanini G, Montanari C, Casiraghi MC, Ottaviano E, Morace G, et al. Proteobacteria overgrowth and butyrate-producing taxa depletion in the gut microbiota of glycogen storage disease type 1 patients. *Metabolites* (2020) 10:133. doi: 10.3390/metabo10040133

26. Mikami M, Arai A, Mizumoto H. Empagliflozin ameliorated neutropenia in a girl with glycogen storage disease ib. *Pediatr Int* (2021) 63:1394–6. doi: 10.1111/ped.14629

27. Kaczor M, Greczan M, Kierus K, Ehmke vel Emczyńska-Seliga E, Ciara E, Piątosa B, et al. Sodium-glucose cotransporter type 2 channel inhibitor: breakthrough in the treatment of neutropenia in patients with glycogen storage disease type 1b? *JIMD Rep* (2022) 63:199–206. doi: 10.1002/jmd2.12278

28. Grünert SC, Rosenbaum-Fabian S, Schumann A, Selbitz A, Merz W, Gieselmann A, et al. Two successful pregnancies and first use of empagliflozin during pregnancy in glycogen storage disease type ib. *JIMD Rep* (2022) 63:303–8. doi: 10.1002/jmd2.12295

29. Halligan RK, Dalton RN, Turner C, Lewis KA, Mundy HR. Understanding the role of SGLT2 inhibitors in glycogen storage disease type ib: the experience of one UK centre. *Orphanet J Rare Dis* (2022) 17:195. doi: 10.1186/s13023-022-02345-2

30. Bidiuk J, Gaciong Z, Sobieraj P. The overall benefits of empagliflozin treatment in adult siblings with glycogen storage disease type ib: one year experience. *Arch Med Sci* (2022) 18:1095–9. doi: 10.5114/aoms/150029

31. Hexner-Erlichman Z, Veiga-da-Cunha M, Zehavi Y, Vadasz Z, Sabag AD, Tatour S, et al. Favorable outcome of empagliflozin treatment in two pediatric glycogen storage disease type 1b patients. *Front Pediatr* (2022) 10:1071464. doi: 10.3389/fped.2022.1071464

32. Tallis E, Karsenty CL, Grimes AB, Karam LB, Elsea SH, Sutton VR, et al. Untargeted metabolomic profiling in a patient with glycogen storage disease ib receiving empagliflozin treatment. *JIMD Rep* (2022) 63:309–15. doi: 10.1002/jmd2.12304

33. Mathis T, Poms M, Köfeler H, Gautschi M, Plecko B, Baumgartner MR, et al. Untargeted plasma metabolomics identifies broad metabolic perturbations in glycogen storage disease type I. *J Inherit Metab Dis* (2022) 45:235–47. doi: 10.1002/jimd.12451

34. Grünert SC, Derks TGJ, Adrian K, Al-Thihli K, Ballhausen D, Bidiuk J, et al. Efficacy and safety of empagliflozin in glycogen storage disease type ib: data from an international questionnaire. *Genet Med* (2022) 24:1781–8. doi: 10.1016/j.gim.2022.04.001

35. Hiwarkar P, Bargir U, Pandrowala A, Bodhanwala M, Thakker N, Taur P, et al. SLGT2 inhibitor rescues myelopoiesis in G6PC3 deficiency. *J Clin Immunol* (2022) 42:1653–9. doi: 10.1007/s10875-022-01323-4

36. Yu S. The anhydrofructose pathway of glycogen catabolism. *IUBMB Life* (2008) 60:798–809. doi: 10.1002/iub.125

37. Ferrannini E, Natali A, Camastra S, Nannipieri M, Mari A, Adam K-P, et al. Early metabolic markers of the development of dysglycemia and type 2 diabetes and their physiological significance. *Diabetes* (2013) 62:1730–7. doi: 10.2337/db12-0707

38. Chen LH, Leung PS. Inhibition of the sodium glucose co-transporter-2: its beneficial action and potential combination therapy for type 2 diabetes mellitus. *Diabetes Obes Metab* (2013) 15:392–402. doi: 10.1111/dom.12064

39. Heerspink HJL, Perkins BA, Fitchett DH, Husain M, Cherney DZI. Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus. *Circulation* (2016) 134:752–72. doi: 10.1161/CIRCULATIONAHA.116.021887

40. Chatterjee S, Khunti K, Davies MJ. Optimizing management of glycaemia. *Best Pract Res Clin Endocrinol Metab* (2016) 30:397–411. doi: 10.1016/j.beem.2016.06.002

41. Michel MC, Mayoux E, Vallon V. A comprehensive review of the pharmacodynamics of the SGLT2 inhibitor empagliflozin in animals and humans. *Naunyn Schmiedebergs Arch Pharmacol* (2015) 388:801–16. doi: 10.1007/s00210-015-1134-1

42. Forycka J, Hajdys J, Krzemińska J, Wilczopolski P, Wronka M, Młynarska E, et al. New insights into the use of empagliflozin–a comprehensive review. *Biomedicines* (2022) 10:3294. doi: 10.3390/biomedicines10123294

43. Nakamura K, Miyoshi T, Yoshida M, Akagi S, Saito Y, Ejiri K, et al. Pathophysiology and treatment of diabetic cardiomyopathy and heart failure in patients with diabetes mellitus. *Int J Mol Sci* (2022) 23:3587. doi: 10.3390/ijms23073587

44. Andreadi A, Bellia A, Di Daniele N, Meloni M, Lauro R, Della-Morte D, et al. The molecular link between oxidative stress, insulin resistance, and type 2 diabetes: a target for new therapies against cardiovascular diseases. *Curr Opin Pharmacol* (2022) 62:85–96. doi: 10.1016/j.coph.2021.11.010

45. Abdelkader NF, Elbaset MA, Moustafa PE, Ibrahim SM. Empagliflozin mitigates type 2 diabetes-associated peripheral neuropathy: a glucose-independent effect through AMPK signaling. *Arch Pharm Res* (2022) 45:475–93. doi: 10.1007/s12272-022-01391-5

46. Shimabukuro M. Cardiac adiposity and global cardiometabolic risk new concept and clinical implication. *Circ J* (2009) 73:27–34. doi: 10.1253/circj.CJ-08-1012

47. Maiorana A, Del Bianco C, Cianfarani S. Adipose tissue: a metabolic regulator. potential implications for the metabolic outcome of subjects born small for gestational age (SGA). *Rev Diabetes Stud* (2007) 4:134–46. doi: 10.1900/RDS.2007.4.134

48. Nakamura K, Miyoshi T, Yunoki K, Ito H. Postprandial hyperlipidemia as a potential residual risk factor. *J Cardiol* (2016) 67:335–9. doi: 10.1016/j.jjcc.2015.12.001

49. Yoshida M, Nakamura K, Miyoshi T, Yoshida M, Kondo M, Akazawa K, et al. Combination therapy with pemafibrate (K-877) and pitavastatin improves vascular endothelial dysfunction in dahl/salt-sensitive rats fed a high-salt and high-fat diet. *Cardiovasc Diabetol* (2020) 19:149. doi: 10.1186/s12933-020-01132-2

50. Inzucchi SE, Zinman B, Wanner C, Ferrari R, Fitchett D, Hantel S, et al. SGLT-2 inhibitors and cardiovascular risk: proposed pathways and review of ongoing outcome trials. *Diabetes Vasc Dis Res* (2015) 12:90–100. doi: 10.1177/1479164114559852

51. Spiga R, Marini MA, Mancuso E, Di Fatta C, Fuoco A, Perticone F, et al. Uric acid is associated with inflammatory biomarkers and induces inflammation Via activating the NF-κB signaling pathway in HepG2 cells. *Arterioscler Thromb Vasc Biol* (2017) 37:1241–9. doi: 10.1161/ATVBAHA.117.309128

52. La Grotta R, de Candia P, Olivieri F, Matacchione G, Giuliani A, Rippo MR, et al. Anti-inflammatory effect of SGLT-2 inhibitors via uric acid and insulin. *Cell Mol Life Sci* (2022) 79:273. doi: 10.1007/s00018-022-04289-z

53. Dong M, Chen H, Wen S, Yuan Y, Yang L, Xu D, et al. The mechanism of sodium-glucose cotransporter-2 inhibitors in reducing uric acid in type 2 diabetes mellitus. *Diabetes Metab Syndr Obes* (2023) 16:437–45. doi: 10.2147/DMSO.S399343

54. Mauer J, Chaurasia B, Plum L, Quast T, Hampel B, Blüher M, et al. Myeloid cell-restricted insulin receptor deficiency protects against obesity-induced inflammation and systemic insulin resistance. *PloS Genet* (2010) 6:e1000938. doi: 10.1371/journal.pgen.1000938

55. Aljada A, Ghanim H, Saadeh R, Dandona P. Insulin inhibits NFκB and MCP-1 expression in human aortic endothelial cells. *J Clin Endocrinol Metab* (2001) 86:450–3. doi: 10.1210/jcem.86.1.7278

56. Borghi C, Bragagni A. The new type 2 diabetes mellitus therapy: comparison between the two classes of drugs GLPR (glucagon-like peptide receptor) agonists and SGLT2 (sodium–glucose cotransporter 2) inhibitors. *Eur Hear J Suppl* (2020) 22:L28–32. doi: 10.1093/eurheartj/suaa129

57. Packer M, Anker SD, Butler J, Filippatos G, Pocock SJ, Carson P, et al. Cardiovascular and renal outcomes with empagliflozin in heart failure. *N Engl J Med* (2020) 383:1413–24. doi: 10.1056/NEJMoa2022190

58. Williams DM, Evans M. Are SGLT-2 inhibitors the future of heart failure treatment? the EMPEROR-preserved and EMPEROR-reduced trials. *Diabetes Ther* (2020) 11:1925–34. doi: 10.1007/s13300-020-00889-9

59. Santer R, Calado J. Familial renal glucosuria and SGLT2: from a mendelian trait to a therapeutic target. *Clin J Am Soc Nephrol* (2010) 5:133–41. doi: 10.2215/CJN.04010609

60. Packer M. SGLT2 inhibitors produce cardiorenal benefits by promoting adaptive cellular reprogramming to induce a state of fasting mimicry: a paradigm shift in understanding their mechanism of action. *Diabetes Care* (2020) 43:508–11. doi: 10.2337/dci19-0074

61. Nakamura M, Sadoshima J. Cardiomyopathy in obesity, insulin resistance and diabetes. *J Physiol* (2020) 598:2977–93. doi: 10.1113/JP276747

62. Volpe CMO, Villar-Delfino PH, dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. *Cell Death Dis* (2018) 9:119. doi: 10.1038/s41419-017-0135-z

63. Koyani CN, Plastira I, Sourij H, Hallström S, Schmidt A, Rainer PP, et al. Empagliflozin protects heart from inflammation and energy depletion via AMPK activation. *Pharmacol Res* (2020) 158:104870. doi: 10.1016/j.phrs.2020.104870

64. Lu Q, Liu J, Li X, Sun X, Zhang J, Ren D, et al. Empagliflozin attenuates ischemia and reperfusion injury through LKB1/AMPK signaling pathway. *Mol Cell Endocrinol* (2020) 501:110642. doi: 10.1016/j.mce.2019.110642

65. Zhou H, Wang S, Zhu P, Hu S, Chen Y, Ren J. Empagliflozin rescues diabetic myocardial microvascular injury via AMPK-mediated inhibition of mitochondrial fission. *Redox Biol* (2018) 15:335–46. doi: 10.1016/j.redox.2017.12.019

66. Zheng H, Zhu H, Liu X, Huang X, Huang A, Huang Y. Mitophagy in diabetic cardiomyopathy: roles and mechanisms. *Front Cell Dev Biol* (2021) 9:750382. doi: 10.3389/fcell.2021.750382

67. Kato ET, Kimura T. Sodium-glucose Co-transporters-2 inhibitors and heart failure: state of the art review and future potentials. *Int J Hear Fail* (2020) 2:12. doi: 10.36628/ijhf.2019.0013

68. Zelniker TA, Wiviott SD, Raz I, Im K, Goodrich EL, Bonaca MP, et al. SGLT2 inhibitors for primary and secondary prevention of cardiovascular and renal outcomes in type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet* (2019) 393:31–9. doi: 10.1016/S0140-6736(18)32590-X

69. Das NA, Carpenter AJ, Belenchia A, Aroor AR, Noda M, Siebenlist U, et al. Empagliflozin reduces high glucose-induced oxidative stress and miR-21-dependent TRAF3IP2 induction and RECK suppression, and inhibits human renal proximal tubular epithelial cell migration and epithelial-to-mesenchymal transition. *Cell Signal* (2020) 68:109506. doi: 10.1016/j.cellsig.2019.109506

70. Quagliariello V, De Laurentiis M, Rea D, Barbieri A, Monti MG, Carbone A, et al. The SGLT-2 inhibitor empagliflozin improves myocardial strain, reduces cardiac fibrosis and pro-inflammatory cytokines in non-diabetic mice treated with doxorubicin. *Cardiovasc Diabetol* (2021) 20:150. doi: 10.1186/s12933-021-01346-y

71. Zeng S, Delic D, Chu C, Xiong Y, Luo T, Chen X, et al. Antifibrotic effects of low dose SGLT2 inhibition with empagliflozin in comparison to ang II receptor blockade with telmisartan in 5/6 nephrectomised rats on high salt diet. *BioMed Pharmacother* (2022) 146:112606. doi: 10.1016/j.biopha.2021.112606

72. Santos-Ferreira D, Gonçalves-Teixeira P, Fontes-Carvalho R. SGLT-2 inhibitors in heart failure and type-2 diabetes: hitting two birds with one stone? *Cardiology* (2020) 145:311–20. doi: 10.1159/000504694

73. Cherney DZI, Perkins BA, Soleymanlou N, Maione M, Lai V, Lee A, et al. Renal hemodynamic effect of sodium-glucose cotransporter 2 inhibition in patients with type 1 diabetes mellitus. *Circulation* (2014) 129:587–97. doi: 10.1161/CIRCULATIONAHA.113.005081

74. Zelniker TA, Braunwald E. Cardiac and renal effects of sodium-glucose Co-transporter 2 inhibitors in diabetes: JACC state-of-the-Art review. *J Am Coll Cardiol* (2018) 72:1845–55. doi: 10.1016/j.jacc.2018.06.040

75. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. *N Engl J Med* (2015) 373:2117–28. doi: 10.1056/NEJMoa1504720

76. Packer M, Butler J, Zannad F, Filippatos G, Ferreira JP, Pocock SJ, et al. Effect of empagliflozin on worsening heart failure events in patients with heart failure and preserved ejection fraction: EMPEROR-preserved trial. *Circulation* (2021) 144:1284–94. doi: 10.1161/CIRCULATIONAHA.121.056824

77. Packer M, Butler J, Zannad F, Pocock SJ, Filippatos G, Ferreira JP, et al. Empagliflozin and major renal outcomes in heart failure. *N Engl J Med* (2021) 385:1531–3. doi: 10.1056/NEJMc2112411

78. Wanner C. EMPA-REG OUTCOME: the nephrologist's point of view. *Am J Cardiol* (2017) 120:S59–67. doi: 10.1016/j.amjcard.2017.05.012

79. Kelly MS, Lewis J, Huntsberry AM, Dea L, Portillo I. Efficacy and renal outcomes of SGLT2 inhibitors in patients with type 2 diabetes and chronic kidney disease. *Postgrad Med* (2019) 131:31–42. doi: 10.1080/00325481.2019.1549459

80. Braunwald E. SGLT2 inhibitors: the statins of the 21stcentury. *Eur Heart J* (2022) 43:1029–30. doi: 10.1093/eurheartj/ehab765

81. Sizar O, Podder V, Talati R. *Empagliflozin*. 2215 Constitution Avenue, N.W. Washington, DC: The American Pharmacists Association (2022) p. 45–47 p. doi: 10.21019/druginformation.empagliflozin

82. Cherney D, Lund SS, Perkins BA, Groop P-H, Cooper ME, Kaspers S, et al. The effect of sodium glucose cotransporter 2 inhibition with empagliflozin on microalbuminuria and macroalbuminuria in patients with type 2 diabetes. *Diabetologia* (2016) 59:1860–70. doi: 10.1007/s00125-016-4008-2

83. American Diabetes Association. 10. cardiovascular disease and risk management: standards of medical care in diabetes–2021. *Diabetes Care* (2021) 44: S125–50. doi: 10.2337/dc21-S010

84. Szrejder M, Piwkowska A. AMPK signalling: implications for podocyte biology in diabetic nephropathy. *Biol Cell* (2019) 111:109–20. doi: 10.1111/boc.201800077

85. Kitada M, Kume S, Takeda-Watanabe A, Kanasaki K, Koya D. Sirtuins and renal diseases: relationship with aging and diabetic nephropathy. *Clin Sci* (2013) 124:153–64. doi: 10.1042/CS20120190

86. Takahashi A, Takabatake Y, Kimura T, Maejima I, Namba T, Yamamoto T, et al. Autophagy inhibits the accumulation of advanced glycation end products by promoting lysosomal biogenesis and function in the kidney proximal tubules. *Diabetes* (2017) 66:1359–72. doi: 10.2337/db16-0397

87. Umino H, Hasegawa K, Minakuchi H, Muraoka H, Kawaguchi T, Kanda T, et al. High basolateral glucose increases sodium-glucose cotransporter 2 and reduces sirtuin-1 in renal tubules through glucose transporter-2 detection. *Sci Rep* (2018) 8:6791. doi: 10.1038/s41598-018-25054-y

88. Lee YH, Kim SH, Kang JM, Heo JH, Kim D-J, Park SH, et al. Empagliflozin attenuates diabetic tubulopathy by improving mitochondrial fragmentation and autophagy. *Am J Physiol Physiol* (2019) 317:F767–80. doi: 10.1152/ajprenal.00565.2018

89. Hardy T, Oakley F, Anstee QM, Day CP. Nonalcoholic fatty liver disease: pathogenesis and disease spectrum. *Annu Rev Pathol Mech Dis* (2016) 11:451–96. doi: 10.1146/annurev-pathol-012615-044224

90. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* (2018) 15:11–20. doi: 10.1038/nrgastro.2017.109

91. Caussy C, Aubin A, Loomba R. The relationship between type 2 diabetes, NAFLD, and cardiovascular risk. *Curr Diabetes Rep* (2021) 21:15. doi: 10.1007/s11892-021-01383-7

92. Dogru T, Kirik A, Gurel H, Rizvi AA, Rizzo M, Sonmez A. The evolving role of fetuin-a in nonalcoholic fatty liver disease: an overview from liver to the heart. *Int J Mol Sci* (2021) 22:6627. doi: 10.3390/ijms22126627

93. Mori K, Emoto M, Inaba M. Fetuin-a: a multifunctional protein. *Recent Pat Endocr Metab Immune Drug Discov* (2011) 5:124–46. doi: 10.2174/187221411799015372

94. Lee K-Y, Lee W, Jung S-H, Park J, Sim H, Choi Y-J, et al. Hepatic upregulation of fetuin-a mediates acetaminophen-induced liver injury through activation of TLR4 in mice. *Biochem Pharmacol* (2019) 166:46–55. doi: 10.1016/j.bcp.2019.05.011

95. Hüttl M, Markova I, Miklankova D, Zapletalova I, Poruba M, Haluzik M, et al. In a prediabetic model, empagliflozin improves hepatic lipid metabolism independently of obesity and before onset of hyperglycemia. *Int J Mol Sci* (2021) 22:11513. doi: 10.3390/ijms222111513

96. Ashino T, Ohkubo-Morita H, Yamamoto M, Yoshida T, Numazawa S. Possible involvement of nuclear factor erythroid 2-related factor 2 in the gene expression of Cyp2b10 and Cyp2a5. *Redox Biol* (2014) 2:284–8. doi: 10.1016/j.redox.2013.12.025

97. Guerra S, Gastaldelli A. The role of the liver in the modulation of glucose and insulin in non alcoholic fatty liver disease and type 2 diabetes. *Curr Opin Pharmacol* (2020) 55:165–74. doi: 10.1016/j.coph.2020.10.016

98. Petersen MC, Shulman GI. Roles of diacylglycerols and ceramides in hepatic insulin resistance. *Trends Pharmacol Sci* (2017) 38:649–65. doi: 10.1016/j.tips.2017.04.004

99. Lebeaupin C, Vallée D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J Hepatol* (2018) 69:927–47. doi: 10.1016/j.jhep.2018.06.008

100. Pokharel A KCS, Thapa P, Karki N, Shrestha R, Jaishi B, Paudel MS. The effect of empagliflozin on liver fat in type 2 diabetes mellitus patients with non-alcoholic fatty liver disease. *Cureus* (2021) 2. doi: 10.7759/cureus.16687

101. Bellanti F, Lo BA, Dobrakowski M, Kasperczyk A, Kasperczyk S, Aich P, et al. Impact of sodium glucose cotransporter-2 inhibitors on liver steatosis/fibrosis/inflammation and redox balance in non-alcoholic fatty liver disease. *World J Gastroenterol* (2022) 28:3243–57. doi: 10.3748/wjg.v28.i26.3243

102. Duby JJ, Campbell RK, Setter SM, White JR, Rasmussen KA. Diabetic neuropathy: an intensive review. *Am J Heal Pharm* (2004) 61:160–73. doi: 10.1093/ajhp/61.2.160

103. Obrosova IG. How does glucose generate oxidative stress in peripheral nerve? *Int Rev Neurobiol* (2002), 3–35. doi: 10.1016/S0074-7742(02)50071-4

104. Vincent AM, Russell JW, Low P, Feldman EL. Oxidative stress in the pathogenesis of diabetic neuropathy. *Endocr Rev* (2004) 25:612–28. doi: 10.1210/er.2003-0019

105. Fernyhough P, Schmidt RE. Neurofilaments in diabetic neuropathy. *Int Rev Neurobiol* (2002), 115–44. doi: 10.1016/S0074-7742(02)50075-1

106. Roy Chowdhury SK, Smith DR, Saleh A, Schapansky J, Marquez A, Gomes S, et al. Impaired adenosine monophosphate-activated protein kinase signalling in dorsal root ganglia neurons is linked to mitochondrial dysfunction and peripheral neuropathy in diabetes. *Brain* (2012) 135:1751–66. doi: 10.1093/brain/aws097

107. Yerra VG, Kumar A. Adenosine monophosphate-activated protein kinase abates hyperglycaemia-induced neuronal injury in experimental models of diabetic neuropathy: effects on mitochondrial biogenesis, autophagy and neuroinflammation. *Mol Neurobiol* (2017) 54:2301–12. doi: 10.1007/s12035-016-9824-3

108. Lin C-H, Cheng Y-C, Nicol CJ, Lin K-H, Yen C-H, Chiang M-C. Activation of AMPK is neuroprotective in the oxidative stress by advanced glycosylation end products in human neural stem cells. *Exp Cell Res* (2017) 359:367–73. doi: 10.1016/j.yexcr.2017.08.019

109. Liu S-Y, Chen L, Li X-C, Hu Q-K, He L-J. Lycium barbarum polysaccharide protects diabetic peripheral neuropathy by enhancing autophagy via mTOR/p70S6K inhibition in streptozotocin-induced diabetic rats. *J Chem Neuroanat* (2018) 89:37–42. doi: 10.1016/j.jchemneu.2017.12.011

110. Russell RC, Tian Y, Yuan H, Park HW, Chang Y-Y, Kim J, et al. ULK1 induces autophagy by phosphorylating beclin-1 and activating VPS34 lipid kinase. *Nat Cell Biol* (2013) 15:741–50. doi: 10.1038/ncb2757

111. Steunenberg TAH, Peeks F, Hoogeveen IJ, Mitchell JJ, Mundy H, de Boer F, et al. Safety issues associated with dietary management in patients with hepatic glycogen storage disease. *Mol Genet Metab* (2018) 125:79–85. doi: 10.1016/j.ymgme.2018.07.004

112. Donnan JR, Grandy CA, Chibrikov E, Marra CA, Aubrey-Bassler K, Johnston K, et al. Comparative safety of the sodium glucose co-transporter 2 (SGLT2) inhibitors: a systematic review and meta-analysis. *BMJ Open* (2019) 9:1–15. doi: 10.1136/bmjopen-2018-022577

113. Rosenstock J, Jelaska A, Frappin G, Salsali A, Kim G, Woerle HJ, et al. Improved glucose control with weight loss, lower insulin doses, and no increased hypoglycemia with empagliflozin added to titrated multiple daily injections of insulin in obese inadequately controlled type 2 diabetes. *Diabetes Care* (2014) 37:1815–23. doi: 10.2337/dc13-3055

114. Worth C, Hoskyns L, Salomon-Estebanez M, Nutter PW, Harper S, Derks TG, et al. Continuous glucose monitoring for children with hypoglycaemia: evidence in 2023. *Front Endocrinol (Lausanne)* (2023) 14:1116864. doi: 10.3389/fendo.2023.1116864

115. Rayannavar A, Elci OU, Mitteer L, De León 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

116. Alsaffar H, Turner L, Yung Z, O'Hara C, Didi M, Senniappan S. Flash glucose monitoring in children with congenital hyperinsulinism; first report on accuracy and patient experience. *Endocr Abstr* (2016), 4–9. doi: 10.1530/endoabs.45.P55

117. dos Santos SS, Ramaldes LA, Gabbay MAL, Moises RCS, Dib SA. Use of a sodium-glucose cotransporter 2 inhibitor, empagliflozin, in a patient with rabson-mendenhall syndrome. *Horm Res Paediatr* (2021) 94:313–6. doi: 10.1159/000519613

118. Galderisi A, Tamborlane W, Taylor SI, Attia N, Moretti C, Barbetti F. SGLT2i improves glycemic control in patients with congenital severe insulin resistance. *Pediatrics* (2022) 150:1–4. doi: 10.1542/peds.2021-055671

119. Chen Y-T, Kishnani PS, Koeberl D. Glycogen storage diseases. In: Valle DL, Antonarakis S, Ballabio A, Beaudet AL, Mitchell GA, editors. *The online metabolic and molecular bases of inherited disease*. New York, NY: McGraw-Hill Education (2019). Available at: http://ommbid.mhmedical.com/content.aspx?aid=1181420647.

120. Ng ES-T, Gupta S, Khin SM, Mak A. Gout, anemia, and hepatomegaly in a young man with glycogen storage disease. *JCR J Clin Rheumatol* (2012) 18:222–3. doi: 10.1097/RHU.0b013e3182598ed1

121. Ouchi M, Oba K, Aoyama J, Watanabe K, Ishii K, Yano H, et al. Serum uric acid in relation to serum 1,5-anhydroglucitol levels in patients with and without type 2 diabetes mellitus. *Clin Biochem* (2013) 46:1436–41. doi: 10.1016/j.clinbiochem.2013.06.003

122. Dougherty JA, Guirguis E, Thornby K-A. A systematic review of newer antidiabetic agents in the treatment of nonalcoholic fatty liver disease. *Ann Pharmacother* (2021) 55:65–79. doi: 10.1177/1060028020935105

123. Han JH, Oh TJ, Lee G, Maeng HJ, Lee DH, Kim KM, et al. The beneficial effects of empagliflozin, an SGLT2 inhibitor, on atherosclerosis in ApoE –/– mice fed a western diet. *Diabetologia* (2017) 60:364–76. doi: 10.1007/s00125-016-4158-2

124. Terami N, Ogawa D, Tachibana H, Hatanaka T, Wada J, Nakatsuka A, et al. Long-term treatment with the sodium glucose cotransporter 2 inhibitor, dapagliflozin, ameliorates glucose homeostasis and diabetic nephropathy in db/db mice. *PloS One* (2014) 9:e100777. doi: 10.1371/journal.pone.0100777

125. Udwan K, Abed A, Roth I, Dizin E, Maillard M, Bettoni C, et al. Dietary sodium induces a redistribution of the tubular metabolic workload. *J Physiol* (2017) 595:6905–22. doi: 10.1113/JP274927

126. Huang DY, Gao H, Boini KM, Osswald H, Nürnberg B, Lang F. In vivo Stimulation of AMP-activated protein kinase enhanced tubuloglomerular feedback but reduced tubular sodium transport during high dietary NaCl intake. *Pflügers Arch - Eur J Physiol* (2010) 460:187–96. doi: 10.1007/s00424-010-0803-7

127. Rossi A, Hoogeveen IJ, Bastek VB, 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

128. Massese M, Tagliaferri F, Dionisi-Vici C, Maiorana A. Glycogen storage diseases with liver involvement: a literature review of GSD type 0, IV, VI, IX and XI. *Orphanet J Rare Dis* (2022) 17:1–12. doi: 10.1186/s13023-022-02387-6

129. Ribas GS, Vargas CR. Evidence that oxidative disbalance and mitochondrial dysfunction are involved in the pathophysiology of fatty acid oxidation disorders. *Cell Mol Neurobiol* (2022) 42:521–32. doi: 10.1007/s10571-020-00955-7

130. 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

131. Luciani A, Denley MCS, Govers LP, Sorrentino V, Froese DS. Mitochondrial disease, mitophagy, and cellular distress in methylmalonic acidemia. *Cell Mol Life Sci* (2021) 78:6851–67. doi: 10.1007/s00018-021-03934-3

132. Seranova E, Connolly KJ, Zatyka M, Rosenstock TR, Barrett T, Tuxworth RI, et al. Dysregulation of autophagy as a common mechanism in lysosomal storage diseases. *Essays Biochem* (2017) 61:733–49. doi: 10.1042/EBC20170055

133. Rajas F, Labrune P, Mithieux G. Glycogen storage disease type 1 and diabetes: learning by comparing and contrasting the two disorders. *Diabetes Metab* (2013) 39:377–87. doi: 10.1016/j.diabet.2013.03.002

134. Melis D, Rossi A, Pivonello R, Salerno M, Balivo F, Spadarella S, et al. Glycogen storage disease type ia (GSDIa) but not glycogen storage disease type ib (GSDIb) is associated to an increased risk of metabolic syndrome: possible role of microsomal glucose 6-phosphate accumulation. *Orphanet J Rare Dis* (2015) 10:91. doi: 10.1186/s13023-015-0301-2

135. Rossi A, Simeoli C, Salerno M, Ferrigno R, Della Casa R, Colao A, et al. Imbalanced cortisol concentrations in glycogen storage disease type I: evidence for a possible link between endocrine regulation and metabolic derangement. *Orphanet J Rare Dis* (2020) 15:99. doi: 10.1186/s13023-020-01377-w

136. Rossi A, Ruoppolo M, Formisano P, Villani G, Albano L, Gallo G, et al. Insulin-resistance in glycogen storage disease type ia: linking carbohydrates and mitochondria? *J Inherit Metab Dis* (2018) 41:985–95. doi: 10.1007/s10545-018-0149-4

137. Bhattacharya K. Dietary dilemmas in the management of glycogen storage disease type I. *J Inherit Metab Dis* (2011) 34:621–9. doi: 10.1007/s10545-011-9322-8

138. Dahlberg KR, Ferrecchia IA, Dambska-Williams M, Resler TE, Ross KM, Butler GL, et al. Cornstarch requirements of the adult glycogen storage disease ia population: a retrospective review. *J Inherit Metab Dis* (2020) 43:269–78. doi: 10.1002/jimd.12160

139. Qian H, Chao X, Williams J, Fulte S, Li T, Yang L, et al. Autophagy in liver diseases: a review. *Mol Aspects Med* (2021) 82:100973. doi: 10.1016/j.mam.2021.100973

140. Halaby CA, Young SP, Austin S, Stefanescu E, Bali D, Clinton LK, et al. Liver fibrosis during clinical ascertainment of glycogen storage disease type III: a need for improved and systematic monitoring. *Genet Med* (2019) 21:2686–94. doi: 10.1038/s41436-019-0561-7

141. Gautam S, Zhang L, Lee C, Arnaoutova I, Chen HD, Resaz R, et al. Molecular mechanism underlying impaired hepatic autophagy in glycogen storage disease type ib. *Hum Mol Genet* (2023) 32:262–75. doi: 10.1093/hmg/ddac197

142. Busch V, Gempel K, Hack A, Müller K, Vorgerd M, Lochmüller H, et al. Treatment of glycogenosis type V with ketogenic diet. *Ann Neurol* (2005) 58:341. doi: 10.1002/ana.20565

143. Vorgerd M, Zange J. Treatment of glycogenosys type V (McArdle disease) with creatine and ketogenic diet with clinical scores and with 31P-MRS on working leg muscle. *Acta Myol myopathies cardiomyopathies Off J Mediterr Soc Myol* (2007) 26:61–3.

144. Løkken N, Hansen KK, Storgaard JH, Ørngreen MC, Quinlivan R, Vissing J. Titrating a modified ketogenic diet for patients with McArdle disease: a pilot study. *J Inherit Metab Dis* (2020) 43:778–86. doi: 10.1002/jimd.12223

145. Similä ME, Auranen M, Piirilä PL. Beneficial effects of ketogenic diet on phosphofructokinase deficiency (Glycogen storage disease type VII). *Front Neurol* (2020) 11:57. doi: 10.3389/fneur.2020.00057

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Neonatal hypoglycemia: lack of evidence for a safe management

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Neonatal hypoglycemia affects up to 15% of all newborns. Despite the high prevalence there is no uniform definition of neonatal hypoglycemia, and existing guidelines differ significantly in terms of when and whom to screen for hypoglycemia, and where to set interventional thresholds and treatment goals. In this review, we discuss the difficulties to define hypoglycemia in neonates. Existing knowledge on different strategies to approach this problem will be reviewed with a focus on long-term neurodevelopmental outcome studies and results of interventional trials. Furthermore, we compare existing guidelines on the screening and management of neonatal hypoglycemia. We summarize that evidence-based knowledge about whom to screen, how to screen, and how to manage neonatal hypoglycemia is limited – particularly regarding operational thresholds (single values at which to intervene) and treatment goals (what blood glucose to aim for) to reliably prevent neurodevelopmental sequelae. These research gaps need to be addressed in future studies, systematically comparing different management strategies to progressively optimize the balance between prevention of neurodevelopmental sequelae and the burden of diagnostic or therapeutic procedures. Unfortunately, such studies are exceptionally challenging because they require large numbers of participants to be followed for years, as mild but relevant neurological consequences may not become apparent until mid-childhood or even later. Until there is clear, reproducible evidence on what blood glucose levels may be tolerated without negative impact, the operational threshold needs to include some safety margin to prevent potential long-term neurocognitive impairment from outweighing the short-term burden of hypoglycemia prevention during neonatal period.

KEYWORDS

neonatal hypoglycemia, brain damage, treatment threshold, at-risk neonates, treatment guidelines

Abbreviations: AGA, Appropriate for gestational age; CI, 95% confidence interval; LGA, Large for gestational age; OR, Odds ratio; RR, Risk ratio; SDS, Standard Deviation Score; SGA, Small for gestational age.

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Introduction: neonatal glycemia – what is normal?

For a variety of reasons, the definition of "hypoglycemia" in newborns is more difficult than in adults. In adults, low blood glucose is considered "hypoglycemia" if blood glucose is sufficiently low to cause adrenergic or neuroglycopenic symptoms. Further evaluation or treatment of low glucose values is only recommended if the so-called Whipple's triad is fulfilled (1, 2): 1) the development of autonomic or neuroglycopenic symptoms, 2) a low blood glucose level, and 3), prompt relief of symptoms when blood glucose has risen. As numeric definition, e.g. for clinical studies, a "low glucose" concentration <3.0 mmol/l (<54 mg/dl) was considered a clinically significant hypoglycemia as this is in a range where autonomic and/or neuroglycopenic symptoms are known to occur, however with high intra- and interindividual variability (3).

In newborns, a definition including clinical signs as hallmark cannot be applied, as they are neither specific nor sensitive at this stage of life, even at very low blood glucose levels (4). In addition, there is a physiological, transient drop of blood glucose levels during the first hours and days of life, complicating numeric definitions. Furthermore, the management of blood glucose in the neonatal period does not only need to consider what is "normal", but also what is safe – particularly in terms of adverse neurodevelopmental outcomes, which is still a controversial issue (5–7).

Definition of neonatal hypoglycemia - statistical approach

Quantitative laboratory parameters are usually assessed using population-based reference data, assuming a condition can be considered pathologic if its occurrence is statistically rare, e.g., outside of two standard deviations (~ <2.5th or >97.5th percentile), or <5th/>95th percentile. From an evolutionary perspective, harmful conditions can be expected to occur rather rarely because an adaptation to the environment has taken place. With respect to neonatal hypoglycemia, an epidemiological approach means that we assume that the postnatal metabolism with respect to neonatal hypoglycemia evolved such that under normal circumstances, only a small number of newborns should exhibit relevant hypoglycemic damage. It may be doubted that this perspective is true for neonatal hypoglycemia, as providing all newborns with the best possible care and thus enabling them to develop as optimally as possible may require different threshold values than a plain statistical definition can offer.

The situation is further complicated by the fact that not only the individual low blood glucose value is important, but also the duration of an episode with low values is relevant for an insufficient energy supply, which affects the newborn and may lead to brain damage. Additionally, repeated episodes of low blood glucose levels, leading to a depletion of the low energy stores in the brain are also not captured by a statistical approach of defining a single glucose value as clinically relevant hypoglycemia. Thus, the pathologic relevance or potential for harm is therefore likely to be a continuum and not dichotomous, with mild hypoglycemia having limited or no impact and the risk of adverse consequences gradually increasing with the severity and duration of hypoglycemia (8, 9). Finally, it remains unclear whether a normal range for healthy-term newborns can be extrapolated to newborns at-risk.

A statistical definition must take into account that postnatal blood glucose levels are crucially dependent on postnatal food intake (10, 11). Therefore, reference levels also depend on breastfeeding habits, and the availability of breast/formula milk. Ideally, only studies that followed reasonable postnatal breastfeeding or formula feeding practices in the first hours of life should be considered as reference data, as these are more reflective of how blood glucose *should* be with adequate postnatal feeding.

For healthy term newborns, there are different data on the course of glucose concentrations during the first days of life. Srinivasan et al. published the blood glucose concentrations of 344 healthy full term appropriate for gestational age (AGA) infants. The lower estimate for the 95% confidence interval (CI) was 1.4 mmol/l (26 mg/dl) at one hour of life, increasing to 2.3 mmol/l (42 mg/dl) at two hours. Already at 3 hours of age, glucose concentration was significantly higher than at 1 hour of age, even without initiating of feeding. The authors concluded that glucose levels <1.9 mmol/l (<35 mg/dl) should be of concern in the first 3 hours of life, <2.2 mmol/l (<40 mg/dl) from 3-24 hours of life, and <2.5 mmol/l (<45 mg/dl) subsequently (12).

In contrast, a study from India found no significant variation in the blood glucose concentration between 3 and 72 hours of life in 200 healthy term neonates. Low glucose concentration was defined as <2.2 mmol/l (<40 mg/dl) for the first 24 hours of life and <2.5 mmol/l (<45 mg/dl) at 24-72 hours of life. Of note, the average birth weight of the cohort was only 2650 g, which is significantly lower than the average birth weight of infants of European decent, and all infants were exclusively and frequently breastfed at intervals of 1.5 to 2 hour from 6 hours of life. No differences in glucose concentration were observed with respect to time since last breastfeeding with this regimen. In 27 of 800 measurements, glucose concentrations were below 1.6 mmol/l (29 mg/dl) but higher than 1.3 mmol/l (23 mg/dl). Only one measurement revealed a level <1.3 mmol/l (<23mg/dl), and all infants were asymptomatic and attained "euglycemic levels" after feeding. Of note, low glucose levels <1.6 mmol/l (<29 mg/dl) were noted primarily around the 3rd hour of life but also after 72 hours of life, and a low glucose at 3 hours increased the risk for low glucose at 72 hours (risk ratio (RR) 6.55 [95% CI 3.93 – 10.92], $p < 0.00001$) (13). Alkalay et al. pooled the data of six selected studies and defined thresholds of low blood glucose corresponding to the 5th percentile for different time intervals based on 723 term newborns. The lower estimated <5th percentiles were <1.6 mmol/l (<28 mg/dl) (1-2h); <2.2 mmol/l (<40 mg/dl) (3-23h); <2.3 mmol/l (<41 mg/dl) (24-47h); <2.7 mmol/l (<48 mg/dl) (>48h), and were considered pragmatic operational thresholds, values at which a reaction is recommended (14). The concept of operational thresholds is discussed below.

In a recent study, Harris et al. examined longitudinal neonatal glucose concentrations in 67 healthy, term-born, AGA singletons in New Zealand by continuous glucose monitoring and repeated heel-

prick blood glucose measurements from birth to 120 hours. Most of the infants were exclusively breast-fed. They noted an increase in mean glucose concentration during the first 18 hours, followed by a second phase in which glucose levels remained stable in the same range up to 48 hours, followed by an increase to a new plateau by the fourth day. Blood glucose concentrations of 2.6 mmol/l (47 mg/dl), often used as a threshold, corresponded approximately to the 10^{th} percentile in the first 48 hours. Of the neonates for whom complete interstitial glucose data were available, 73% had at least one episode below this threshold, most within the first 12 hours. Using an interstitial glucose concentration threshold of <2.0 mmol/l (<36 mg/dl), approximately 25% of neonates were found to be below this level at least for one episode. Most of these lower interstitial or blood glucose concentrations occurred within the first 12 hours. There were no episodes of blood glucose concentrations of <1.5 mmol/L (<27 mg/dl) and only two episodes below this cut-off documented by continuous interstitial glucose measurements (15).

From this, despite all limitations as influence of ethnic background, feeding habits, glucose measurement intervals or definition of a "normal" percentile, it can be deduced that single measurements or short episodes of glucose concentrations less than 2.0 mmol/l (36 mg/dl) are "normal" using the statistical approach, while glucose concentrations of <1.3 – 1.5 mmol/l (<23 – 27 mg/dl) should be interpreted as unusual for healthy term newborns".

Approach to define hypoglycemia based on short-term physiological consequences of hypoglycemia (endocrine/metabolic responses, signs/symptoms)

This approach assumes that a low blood glucose should be primarily considered unphysiological if it leads to certain endocrine, metabolic, or neurological consequences/symptoms. Systematic data on counterregulatory mechanisms in neonates are scarce. Stanley et al. found low ketones despite elevated concentrations of fatty acid precursors, indicating limited ketone synthesis capacity in 44 healthy term AGA infants at the end of an eight-hour postnatal fast. Glucose precursors were two to three times higher than those found after the neonatal period, indicating immature gluconeogenesis capacity (9). Similar results were obtained by Harris et al. who found low ketone body concentrations but high lactate values in newborns at low glucose levels (16). However, an alternative hypothesis is that in the fasting situation or low glucose state, the neonatal brain consumes both glucose from gluconeogenesis and ketones to such a high degree that distinct different metabolite concentrations are found than in older fasting children, so capacity of ketogenesis and gluconeogenesis might be underestimated by levels of circulating ketones/glucose. During insufficient glucose supply of the brain, the glycogen of the astrocytes is presumably used to supply the neurons, being first converted into lactate, then transported to the neurons to maintain the neuronal function (17). In addition, blood lactate and ketones are taken up by neurons through monocarboxylate transporters (17).

In summary, the energy supply of neurons can be substituted at least in part by other metabolites than glucose. However, the approach of evaluating these metabolic or endocrinological parameters is also not useful in determining at which blood glucose levels hypoglycemia can be defined or brain damage is imminent.

Outcomes of symptomatic hypoglycemia in newborns have been shown to be worse than asymptomatic hypoglycemia (18, 19). However, we have recently shown that clinical signs are unspecific and not sensitive enough to reliably detect neonatal hypoglycemia based on clinical observation. We found a large interobserver differences, and even in the presence of profound hypoglycemia, the sensitivity to detect hypoglycemia based on clinical signs was rather low (4). Furthermore, severe hypoglycemia may present as apathy or coma, which are difficult to distinguish from deep sleep.

Approach to define hypoglycemia based on neurological function or long-term neurological outcome

This approach attempts to find a threshold that allows undisturbed neurological function and development. Values associated with impaired neurological function or neurodevelopment would then be defined as "hypoglycemia".

In 1988, Koh et al. measured sensory evoked potentials as a noninvasive indicator of brain function in relation to blood glucose concentration in 17 children, 5 of whom were newborns. The lowest blood glucose concentration associated with normal neural function in the newborns ranged from 1.9 to 4.2 mmol/l (34 - 76 mg/dl). The blood glucose concentration immediately before the first abnormal evoked potential ranged from 0.7 to 2.5 mmol/l (13 - 45 mg/dl) in these five newborns. The authors suggested that "the blood glucose concentration should be maintained above 2.6 mmol/l (47 mg/dl) to ensure normal neural function in children irrespective of the presence or absence of abnormal clinical signs" (20). As such low proband numbers warrant cautious interpretation, it does not seem appropriate that this study is repeatedly used to justify a hypoglycemia threshold of 2.6 mmol/l (47 mg/dl).

Neuroimaging studies have identified structural cerebral injury associated with severe, recurrent or symptomatic neonatal hypoglycemia, including white matter lesions preferentially in the parieto-occipital lobes, cortical atrophy, changes in the deep grey matter structures of the basal ganglia and thalamus, periventricular lesions, parenchymal hemorrhage, and ischemic strokes (21–30). Recent studies have associated also mild neonatal hypoglycemia <2.6 mmol/l (<47 mg/dl) in otherwise healthy children with a reduced size of deep grey matter brain regions and thinner occipital lobe cortex at the age of 9-10 years, but no differences regarding white matter microstructure were found. Therefore, the authors concluded that deep grey matter regions may be especially vulnerable to the long-term effects of mild neonatal hypoglycemia (31).

Clinical manifestations of hypoglycemic brain injury include cerebral palsy, epilepsy, neurodevelopmental delay and intellectual disability, microcephaly, visual impairment, and hearing deficits

(32). In hypoglycemic disorders such as congenital hyperinsulinism the reported incidence of brain injury due to severe and recurrent hypoglycemia is still as high as 50% (32–36).

While there is no doubt that severe hypoglycemia of any etiology can lead to brain injury, the effects of mild and transitory neonatal hypoglycemia remain unclear.

Adverse clinical outcome after transitory neonatal hypoglycemia was first described 1988 in a larger study of 661 preterm infants with birth weights <1850 g, reporting that blood glucose concentrations <2.6 mmol/l (<47 mg/dl) for five or more days, even if asymptomatic, were associated with serious neurodevelopmental impairment at a corrected age of 18 months (37). Even though this study had limitations, such as including only preterm infants with e.g., immature counterregulatory response to hypoglycemia, the aim to develop a definition of neonatal hypoglycemia became more urgent. As a consequence of the study, a threshold of 2.6 mmol/l (47 mg/dl) has since been considered a target for the treatment of neonatal hypoglycemia by many neonatologists. However, since 1988, several other studies have examined the neurodevelopmental outcome after neonatal hypoglycemia with contradictory results. In 2019, Shah et al. evaluated nine cohort studies involving 4,041 infants with a gestational age >32 weeks in a systematic review. They concluded that there was low-quality evidence that neonatal hypoglycemia <2.6 mmol/l (<47 mg/dl) was associated with a two- to threefold increased risk of visual-motor impairment and executive dysfunction in early childhood (2–5 years), and a twofold increased risk of literacy and numeracy problems in later childhood (6–11 years). Evidence for an increased risk of general cognitive impairment was rated as very low-quality, and no data was found on the outcome in adolescence with prior neonatal hypoglycemia (38).

One study included in the review was the 'Children With Hypoglycemia and Their Later Development (CHYLD) Study', a longitudinal prospective cohort study investigating neurodevelopmental outcomes in moderate to late preterm and term infants born at risk of hypoglycemia and treated to maintain blood glucose concentrations above 2.6 mmol/l (47 mg/dl). While this study found no association between neonatal hypoglycemia <2.6 mmol/l (<47 mg/dl) and an adverse neurological outcome at two years of age (39), neonatal hypoglycemia was correlated with an increased risk of poor executive function (RR 2.32 [95% confidence interval (CI) 1.17 - 4.59] and poor visual motor function (RR 3.67 [95% CI 1.15 - 11.69] at the age of 4.5 years (40). At 9-10 years, there was no significant difference between children with and without neonatal hypoglycemia regarding the incidence of lower educational achievement (47% vs. 48%; adjusted risk difference −2% [95% CI, −11% to 8%]; adjusted RR 0.95 [95% CI, 0.78 to 1.15]) and other secondary endpoints. However, the reported incidence of low performance overall, including educational achievement, fine-motor and visual-motor functions, emotional behavior regulation and executive function, were concerningly high in both groups and over twice as high as rates expected by the investigators. They concluded that the underlying risk factors for neonatal hypoglycemia and the socioeconomic status rather than hypoglycemia itself may play a greater role in the neurodevelopmental outcome than previously assumed (41). Two other recent studies also did not find any relevant adverse outcome associated with neonatal hypoglycemia if treated to maintain blood glucose concentrations above a certain threshold: In a Danish study, neonatal hypoglycemia <1.7 mmol/l (<30 mg/dl), treated to maintain blood glucose concentrations above 2.5 mmol/l (45 mg/dl), was solely associated with lower fine-motor function in boys (β = - 16.4, p = 0.048) compared to healthy siblings at age 6-9 years. The authors did not find any association between neonatal hypoglycemia and cognitive function, general motor function or behavior. However, the full-scale IQ was 3.2 points lower in the case group compared to the normative population and a major limitation was the low number of sibling controls (n=32) (42).

The HypoEXIT (Hypoglycemia–Expectant Monitoring versus Intensive Treatment) trial was a multicenter, randomized, controlled trial, analyzing noninferiority of a lower treatment threshold strategy (<2.0 mmol/l; <36 mg/dl) to the traditional threshold strategy (<2.6 mmol/l; <47 mg/dl). The operational threshold was defined based on two treatment arms: The intensive treatment arm aimed to rapidly achieve blood glucose concentrations >2.6 mmol/l (>47 mg/dl) by increasing the carbohydrate intake by enteral or intravenous glucose supply. In the expectant glucose monitoring arm, oral nutrition was given aiming at glucose concentrations always >2.0 mmol/l (>36 mg/dl). A glucose concentration below this resulted in intensive treatment. If the first low glucose concentration was <2.0 mmol/l (<36 mg/dl), the infant was excluded from the study and received intensive treatment.

Developmental testing using the Bayley Scales of Infant and Toddler Development in 582 children at the age of 18 months did not cross the prespecified noninferiority limit of −7.5 Bayley-test points for the lower threshold group. However, recurrent or severe hypoglycemia was associated with a worse neurological outcome at follow-up. A limitation of the study is certainly that neonates with initial severe hypoglycemia <2.0 mmol/l (<36 mg/dl) were excluded, the very patients most vulnerable to suffer consequences of hypoglycemia. Furthermore, despite the different intervention thresholds, the mean glucose values during the first two days were quite similar in the two comparative groups (3.2 vs. 3.4 mmol/l; 57 mg/dl vs. 61 mg/dl; mean difference -4.4 [-5.6 to -3.1] (43). Another important limitation is that the patients were quite young at neurodevelopmental follow-up and the long-term data on neurological outcome are not yet available.

Despite these data indicating non-inferior outcome of neonates who suffered neonatal hypoglycemia between 2.0 - 2.6 mmol/l (36 - 47 mg/dl), there are contradicting data. Kaiser et al. matched perinatal data from 1395 children, who had received a universal glucose screening after birth, to their Arkansas Department of Education's fourth-grade achievement test scores. Three cut-offs for hypoglycemia were used (<1.9, <2.2, and <2.5 mmol/l; <35, <40, and <45 mg/dl) and data were controlled for covariables. Even one single hypoglycemic episode <2.5 mmol/l (<45 mg/dl) was associated with decreased school proficiency in literacy (odds ratio (OR) 0.62 [95% CI 0.45 - 0.85] and episodes < 2.2 mmol/l (<40 mg/dl) were associated with decreased proficiency in mathematics (OR 0.51 [95% CI 0.34 – 0.78] at the age of 10 years (44).

In a retrospective population-based study published in 2018, register data of all 101,060 healthy singletons born in two Swedish counties over a period of 4.5 years were screened using International Classification of Diseases 10 (ICD-10) codes, linking a diagnosis of transitory hypoglycemia <2.2 mmol/l (<40 mg/dl) to

prespecified neurologic or developmental diagnoses. The OR of any adverse neurological or neurodevelopmental outcome was 1.48 [95% CI 1.17 - 1.88] in hypoglycemic compared to euglycemic newborns. Furthermore, those with a history of neonatal hypoglycemia had an almost doubled risk of motor delay (OR 1.91 [95% Cl 1.06 - 3.44] and a tripled risk of cognitive developmental delay (OR 3.17 [95% 1.35 - 7.43] (45). In summary, there is also insufficient evidence and too much controversy to define neonatal hypoglycemia based on neurodevelopmental outcome alone. The most valid data available at this time are from the CHYLD study (39–41) as well as the HypoEXIT trial (43), but further studies are needed.

How to integrate different approaches to define hypoglycemia - the operational threshold

Thirty-five years after the publication by Lucas et al. (37), there are still no data available that define how low a glucose concentration must be, respectively how long it must persist at which level to cause brain injury in neonates. Thus, the concept of an "operational threshold" was proposed by Cornblath et al. (46). Operational thresholds should be single blood glucose concentrations at which therapeutic interventions are recommended, to prevent at least those blood glucose concentrations that have a clinically relevant *probability* of causing harm. As such, it will always imply some degree of over- and undertreatment, which needs to be balanced, and does not necessarily follow strict statistical evidence. An operational threshold may be different from a "treatment target", that might be higher and both values may imply some safety margin to reliably prevent blood glucose concentration at which "organ damage is known to occur" (47). Furthermore, the interventional threshold does not need be based on blood glucose levels alone but may also be a composite of blood glucose levels, clinical signs, risk factors for hypoglycemia, presence of symptoms, fasting or satiety state, presumed neurological consequences etc., all of which contribute to therapeutic/interventional decisions. The following section outlines how the approach and handling of different operational thresholds varies in guidelines. These operational thresholds need to be further evaluated in future studies to provide evidence for the recommended interventions so that they are not based solely on expert opinion but rather on research data.

Practical application of the operational threshold: existing guidelines for detection and management of neonatal hypoglycemia

In 1988, Koh et al. found that there was no uniform definition of neonatal hypoglycemia among established pediatric textbooks and or neonatologists in the UK, ranging from a glucose concentration of <1 to <4 mmol/l (<18 to <72 mg/dl). He concluded that this certainly lead to confusion among junior medical and nursing staff and inconsistency in the management of neonatal glycemia (48). In 2019, there continued to be large differences in knowledge about prevention, screening and management of neonatal hypoglycemia among midwives and nurses in Germany (49). Until today, there is still no uniform international guideline for detection and management of neonatal hypoglycemia, however, national guidelines exist in several countries. While some even have multiple guidelines, as for example Australia (50–52), other countries such as Germany do not have a general guideline for neonatal hypoglycemia, but only one for the management of children of diabetic mothers (53). Most guidelines admit that they are based on poor evidence and that there is no consensus on the definition of hypoglycemia and operational thresholds (5, 50, 51, 54–60). UNICEF (United Kingdom) has published a document called "Guidance on the development of policies and guidelines for the prevention and management of hypoglycaemia of the newborn" (61). showing that the topic is highly relevant and some of the existing guidelines refer to this publication as well (54, 56, 60, 62). An exemplary list of published guidelines showing the varying recommendations on who should be screened, when the first blood glucose should be measured, how hypoglycemia is defined, etc. is shown in Table 1. The comparability of glucose values is complicated by the fact that some guidelines refer to blood glucose and others to plasma glucose. For unification, we refer to blood glucose in this manuscript.

Existing guidelines: preventive measures

While most guidelines place a clear focus on preventive measures to avoid hypoglycemia, there are also guidelines that do not address this (5). The majority however recommend initiating breastfeeding/feeding as early as possible (51, 52, 54–60, 62, 63). In addition, some emphasize that mothers should receive adequate guidance and support for breastfeeding and breastfeeding/feeding should be assessed regularly (51, 54, 55, 60, 62). Some guidelines recommend keeping intervals between feedings below three hours (51, 52, 54, 55, 57, 58, 60, 62) while others recommend breastfeeding ad libitum as long as no relevant hypoglycemia occurs (56, 59). Supplemental feeding with formular milk is recommended by Wackernagel et al. for a subgroup of infants in risk (e.g. preterm infants 35 + 0-3 + 6 weeks of gestation, small for gestational age (SGA), large for gestational age (LGA) with maternal diabetes, maternal diabetes not controlled by diet and sick infants in the NICU) and they recommend to use cup feeding or tube feeding instead of bottle feeding if possible (55). The Swiss Society of Neonatology Guideline also recommends offering formula milk immediately after breastfeeding until the mother has enough breast milk to sufficiently feed the neonate, and a prophylactic dose of buccal dextrose gel at one hour after birth (57). Oral dextrose gel (40%) might reduce the need for intravenous fluids in at-risk neonates and decrease NICU admissions with asymptomatic hypoglycemia (64–66), depending on the

TABLE 1 Comparison of international guidelines for screening and management of neonatal hypoglycemia.

	Screening recommended		Time of first BG	Hypoglycemia threshold <48/72 h of life	Management based on asymptomatic/ symptomatic?	Start of i.v. glucose	Further measures included	End of BG screening
	Neonatal risk factors	Maternal risk factors						
Hypoglycemia -newborn, Maternity and Neonatal Clinical Guideline (06/2022, Queensland, Australia) (51)	- Preterm infants (GA <37 weeks) - Postmature infants (GA >42 weeks) - SGA (<10th centile) or BW <2500 g - LGA (>90th centile) or macrosomia - Hypothermia: <36.5 °C or labile - Inadequate feeding - Resuscitation at birth - Polycythemia - Meconium aspiration syndrome - Suspected syndromes - Symptomatic	- Infants of mothers with diabetes - Maternal beta-blockers, dexamethasone, oral hypoglycemics - Family history of metabolic and/or endocrine disorders	Before the 2nd feed (not longer than 3 h of age)	<48 h: <47 mg/dl (<2.6 mmol/l)	Yes	<27 mg/dl (<1.5 mmol/l) or symptomatic	Buccal glucose gel 40% Glucagon i.v. (also continuously)	BG ≥47 mg/dl (≥2.6 mmol/l) for 24 h in 1st 48 h or ≥60 mg/dl (≥3.3) after 48 h
Child Women & Family Services Special Care Baby Unit, Waitemata District Health Board (07/2021, New Zealand) (62)	- Preterm infants (GA <37 weeks) - BW <2.5 kg or SGA (BW <10th centile) - BW >4.5 kg - Hypothermia - Apgar score <7 at 5 minutes - Neonates with hemolytic disease - Neonatal syndromes (e.g., BWS) - All infants with clinical signs - In SCBU, at least daily BG testing on all infants on i.v. fluids	- Diabetes in pregnancy - Maternal drug treatment (e.g., Propranolol, Prozac (Fluoxetine), illicit drug abuse)	At 1-2 h of age	<47 mg/dl (<2.6 mmol/l) (time range not defined)	No	<31 mg/dl (<1.7 mmol/l) 31-45 mg/dl (1.7-2.5 mmol/l): Feeding or i.v. dextrose 10%	Buccal dextrose gel Glucagon	If feeding well, monitor at least for 12 h. Any recorded hypoglycemia, monitor glucose for at least 12 h after last low level
BM Clinical Protocol #1: Guidelines for Glucose Monitoring and Treatment of Hypoglycemia in Term and Late Preterm Neonates (04/2021, U.S.) (56)	- Preterm infants (GA <35 weeks or late preterm infants with clinical signs or extremely poor feeding) - IUGR or marked wasting - BW <2.500 g or SGA <10th centile for weight - Clinically evident wasting of fat + muscle bulk - LGA (>90th centile & macrosomic appearance) - Discordant twin; weight 10% <larger twin - Perinatal stress; severe acidosis or hypoxia–ischemia - Cold stress - Polycythemia - Erythroblastosis fetalis - BWS	- Maternal diabetes or abnormal result of glucose tolerance test, especially if poorly controlled - Pre-eclampsia and pregnancy induced, or essential hypertension - Previous macrocosmic infants (as a proxy for undiagnosed diabetes in pregnancy) - Substance abuse - Treatment with beta-agonist tocolytic - Treatment with oral hypoglycemic agents - Late antepartum or intrapartum administration of i.v. glucose	Infants with suspected significant hyperinsulinemia (e.g., poorly controlled maternal diabetes or known genetic hyperinsulinemia: within 60 min after birth. Other risk groups: Before the 2nd feed, or 2-4 h after birth	<45 mg/dl (<2.5 mmol/l) (time range not defined)	Yes	If BG is repeatedly <45 mg/dl (<2.5 mmol/l) despite feedings If the neonate is unable to suck or feedings are not tolerated, avoid forced feedings and begin IV therapy Infants with abnormal clinical signs, or infants with BG levels <20-25mg/d (<1.1-1.4 mmol/l)	Buccal dextrose gel	Monitoring should continue until acceptable pre-prandial levels are consistently obtained (until the infant has had at least 3 satisfactory BG). A reasonable (although arbitrary) goal is to maintain BG concentrations ≥45 mg/dl (≥2.5 mmol/l). If energy intake falls, glucose monitoring should be recommenced. Late preterm and SGA infants and babies who have clinical

(Continued)

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TABLE 1 Continued

	Screening recommended		Time of first BG	Hypoglycemia threshold <48/72 h of life	Management based on asymptomatic/ symptomatic?	Start of i.v. glucose	Further measures included	End of BG screening
	Neonatal risk factors	Maternal risk factors						
	- Microphallus or midline defect (indicating an underlying endocrine condition) - Suspected infection - Respiratory distress - Known or suspected inborn errors of metabolism or endocrine disorders - Any infant admitted to the NICU - Clinical signs of hypoglycemia							features of intrauterine growth restriction should be monitored (with decreasing frequency) for 24 h
Guideline Hypoglycemia (02/2021, Western Australia) (52)	- Preterm infants (GA <37 weeks) - SGA (<10th centile) - LGA (>97th centile or >4.5 kg) - Beta-blockers in the 3rd trimester	- Maternal diabetes - Antenatal corticosteroids >34 weeks gestation	Before the 2nd feed (3-4 h of age)	<47 mg/dl (<2.6 mmol/l) (time range not defined)	Yes	<27 mg/dl (1.5 mmol/l) or if BG remains between 27-45 mg/dl (1.5-2.5 mmol/l) despite increased feeds	Glucagon i.m.	If 2 consecutive BG are ≥47 mg/dl (≥2.6mmol/l)
Prevention and treatment of hypoglycemia in neonates with a gestational age from 35 0/7 weeks in maternity wards (09/2020, Switzerland) (57)	- Preterm infants (GA<37 0/7 weeks) - BW <2500 g or <3rd centile - BW >4500 g or >97th centile - Hypothermia <36.5°C - Sick newborn infants (e.g., asphyxia, sepsis, respiratory distress, hemolysis) - Symptomatic	- Maternal diabetes (including both, women treated only with dietary intervention and those receiving insulin)	Before the 2nd feed (age 3-4 h)	<47 mg/dl (<2.6 mmol/l) (time range not defined)	No	Not described in detail ➔ only contact neonatal unit	Dextrose gel 40%	If 3 consecutive BG are normal, further blood tests may be discontinued
South Australian Perinatal Practice Guideline, Neonatal Hypoglycemia (09/2020, South Australia) (50)	- Preterm infants (GA ≥32 weeks) - BW <10th centile - BW >97th centile - Transient tachypnea of the newborn - Hypothermia <36.0°C - Suspected asphyxia requiring IPPV or APGAR <6 at 5 min or pH <7.1	- Maternal diabetes - Maternal beta-blockers or valproate	At 1 h of age	1-4 h: ≤36 mg/dl (≤2.0 mmol/l) 5-48 h: <47 mg/dl (<2.6 mmol/l)	Yes, but only for severe symptoms (seizures, critically unwell etc.)	<27 mg/dl (<1.5 mmol/l) <47 mg/dl (<2.6 mmol/l) with other major illness (e.g., seizures, critically unwell baby, severe respiratory distress, suspected infection with clinical instability or heart problem with cyanosis/ poor perfusion or asphyxia with advanced resuscitation)	Buccal glucose gel Glucagon bolus i.m.	After 4 h if BG >36 mg/dl (>2.0 mmol/l) at both 1 and 4 h

(Continued)

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TABLE 1 Continued

	Screening recommended		Time of first BG	Hypoglycemia threshold <48/72 h of life	Management based on asymptomatic/ symptomatic?	Start of i.v. glucose	Further measures included	End of BG screening
	Neonatal risk factors	Maternal risk factors						
Hypoglycemia of the newborn, Women's Health Service, Christchurch Women's (07/2020, New Zealand) (60)	- Preterm infants (GA <37 weeks) - SGA (<9th centile (on UK-WHO growth chart) - LGA (>98thcentile (on UK-WHO growth chart) - Hypothermia - Severe intrapartum fetal distress or lactate >5.8 mmol/L - Asymmetric growth in conjunction with either intrapartum fetal distress and/or meconium exposure - Unwell baby - Sepsis - Symptoms	- Maternal diabetes	pre-feed 3-4 h after birth	<48 h: <47 mg/dl (<2.6 mmol/l)	Yes	Not described in detail only when to contact the neonatal unit	Dextrose gel	One BG <47 mg/dl (<2.6 mmol/l): Until 3 consecutive BG are ≥47 mg/dl (≥2.6 mmol/l) without top-ups or dextrose gel. If a baby has always had BG of 47 mg/dl (2.6 mmol/l) or more and the feeding regime changes, i.e., from breastfeeds with top-ups to fully breastfeeding a pre-feed BG measurement is recommended 6-8 h after the last top-up
Swedish national guideline for prevention and treatment of neonatal hypoglycemia in newborn infants with gestational age ≥ 35 weeks (07/2019, Sweden) (55)	- Preterm infants (GA <37 weeks) - SGA (BW <-2 SDS) - LGA (BW >+2 SDS) - Sick infants at the NICU (e.g., asphyxia, infection) - Clinical signs of hypoglycemia	- Infants of diabetic mothers/mothers with GDM	Before the 2nd feed (not later than 3 h after birth)	<72 h: <47 mg/dl (<2.6 mmol/l)	Yes, but only for severe symptoms (apnea, seizures etc.)	<27 mg/dl (<1.5 mmol/l) or <47 mg/dl (<2.6 mmol/l) and serious symptoms (apnea, seizures, impaired consciousness) or if hypoglycemia persists (27-34 mg/dl) (1.5-1.9 mmol/l) after one (two) meals of supplementary feeds/ dextrose gel	Buccal dextrose gel	Not described in detail
The screening and management of newborns at risk for low blood glucose (05/2019, Canada) (59)	- Preterm infants (GA <37 weeks) - SGA (BW <10th centile) - LGA (BW >90th centile) - Asphyxiated infants	- Infants of mothers with diabetes	At 2 h of age	<72 h: <47 mg/dl (<2.6 mmol/l)	Yes	<32 mg/dl (<1.8 mmol/l) or infants who have failed to respond to enteral supplementation	Dextrose gel	Preterm infants and SGA: after 24 h if BG ≥47 mg/dl (≥2.6 mmol/l) Maternal diabetes and LGA: after 12 h if BG ≥47 mg/dl (≥2.6 mmol/l)

(Continued)

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TABLE 1 Continued

	Screening recommended		Time of first BG	Hypoglycemia threshold <48/72 h of life	Management based on asymptomatic/ symptomatic?	Start of i.v. glucose	Further measures included	End of BG screening
	Neonatal risk factors	Maternal risk factors						
Management of hypoglycemia in newborn: Turkish Neonatal and Pediatric Endocrinology and Diabetes Societies consensus report (2018, Turkey) (58)	- Prematurity - IUGR - SGA - LGA - Hypothermia - Perinatal asphyxia - Meconium aspiration syndrome - Infection - Polycythemia - Drug usage (IV indomethacin) - Immune hemolytic disease (Rh incompatibility) - Congenital heart diseases - Endocrine disorders - Special feature on physical examination findings - History of sibling with hypoglycemia - Malnutrition	- Maternal diabetes - Preeclampsia/ eclampsia, gestation-related hypertension - Medical treatment (beta-blockers, oral hypoglycemic agents, beta-agonist tocolytics, late antepartum and intrapartum dextrose	30 min after first feed	<4 h: ≤40 mg/dl (≤2.2 mmol/l) 4-24 h: ≤45 mg/dl (≤2.5 mmol/l) >24 h: <50 mg/dl (<2.8 mmol/l)	Yes	Symptomatic and <40 mg/dl (<2.2 mmol/l) 0-4 h asymptomatic: BG <25 mg/dl (<1.4 mmol/l) twice despite feeding after first BG <25 mg/dl (<1.4 mmol/l) 4-24 h asymptomatic: BG <35 mg/dl (<1.9 mmol/l) twice despite feeding after first BG <35 mg/dl (<1.9 mmol/l)	Recommendations only if hyperinsulinism is diagnosed	Late preterm (34-36 6/7) and SGA: after 24 h Maternal diabetes and LGA (>34 weeks): after 12 h
Identification and Management of Neonatal Hypoglycemia in the Full Term Infant Framework for Practice, British Association of Perinatal Medicine (04/2017, UK) (54)	- IUGR (BW 2nd centile) or clinically wasted - Perinatal acidosis (cord arterial or infant pH <7.1 and base deficit ≥-12mmol/l) - Hypothermia (<36.5°C) not attributed to environmental factors - Suspected/confirmed early onset sepsis - Abnormal feeding behavior - Clinical signs of hypoglycemia (cyanosis, apnea, altered level of consciousness, seizures, hypotonia, lethargy, high pitched cry)	- Infants of diabetic mothers - Infants of mothers taking beta-blockers in the third trimester and/ or at time of delivery	Before the 2nd feed (2-4 h after birth)	<48 h: <36 mg/dl (<2.0 mmol/l)	Yes	<18 mg/dl (<1.0 mmol/l) and/or clinical signs consistent with hypoglycemia	Buccal dextrose gel Glucagon i.m. (single administration)	BG ≥36 mg/dl (≥2.0 mmol/l) after 3rd measurement (age <8 h). One BG 18-34 mg/dl (1.0-1.9 mmol/l): after 2 consecutive pre-feed BG measurements >2.0 mmol/l and no clinical signs. One BG <18 mg/dl (<1.0 mmol/l): continue to monitor BG until infant is on full enteral feeds and BG values are >45 mg/dl (>2.5 mmol/l) or 54 mg/dl (3.0 mmol/l) in cases of hyperinsulinism over several fast-feed cycles for at least 24 h

(Continued)

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TABLE 1 Continued

	Screening recommended		Time of first BG	Hypoglycemia threshold <48/72 h of life	Management based on asymptomatic/ symptomatic?	Start of i.v. glucose	Further measures included	End of BG screening
	Neonatal risk factors	Maternal risk factors						
Clinical Report-Postnatal Glucose Homeostasis in Late-Preterm and Term Infants, American Academy of Pediatrics (2011, U.S) (5)	- Late preterm infants (GA 34-36 6/7) - SGA - LGA -	- Infants of mothers with diabetes	30 min after first feed	1-4 h: ≤40 mg/dl (≤2.2 mmol/l) 4-24 h: ≤45 mg/dl (≤2.5 mmol/l)	Yes	Symptomatic and BG <40 mg/dl (<2.2 mmol/l). Asymptomatic: Age <4 h: BG <25 mg/dl (<1.4 mmol/l) BG 25-40 mg/dl (1.4-2.2 mmol/l): refeed/i.v. glucose as needed. Age: 4-24 h: BG<35 mg/dl (<1.9 mmol/l). BG 35-45 mg/dl (1.9-2.5 mmol/l): refeed/i.v. glucose as needed.	–	Infants 34-36 6/7 weeks and SGA: after 24 h if BG ≥45 mg/dl (≥2.5 mmol/l). Maternal diabetes and LGA ≥34 weeks: 12 h if BG ≥45 mg/dl (≥2.5 mmol/l).
National guideline to prevent neonatal hypoglycemia (2010, Denmark) (63)	Low risk: - LGA (BW >+2SD*); >+22%) Moderate risk: - SGA (BW<-2SD); <-22%) - IUGR/Immaturity - Preterm (GA 32 + 0 - 36 + 6) - Sepsis, cooling - Light asphyxia (cord-pH 7.0-7.1 or BE -10 to -15) High risk: - Severe asphyxia (cord-pH <7.0 or BE <-15) - Severe IUGR/SGA (BW<-3SD); <-35%)	Low risk: - Diet-treated diabetes mother Moderate risk: - Insulin treated diabetic mother (sufficiently treated) High risk: - Diabetic fetopathies (insulin treated diabetic mother, dysregulated)	Low risk: 2 h Moderate risk: 2 h after first breastfeeding/ nutrition High risk: 1 h old	Depending on risk and age	No	<32 mg/dl (<1.8 mmol/l) Or repeatedly low, depending on the severity and the number of measurements	Diazoxide	Low risk: if first BG ≥2.5 mmol/l

BE, base excess; BWS, Beckwith-Wiedemann syndrome; GA, gestational age; BG, blood glucose; BW, birth weight; SCBU, special care baby unit; SGA, small for gestational age; LGA, large for gestational age; h, hour/s; i.v., intravenous; i.m., intramuscular; NICU, neonatal intensive care unit; SDS, Standard Deviation Score.

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underlying guideline. In addition, an effect on successful implementation of breastfeeding was described (66). However, more data on safety and efficacy and the effect of dextrose gel on long-term neurological outcome are needed. In our view, there is no reliable data on further specific preventive measures. Of note, such measures must also be meaningfully embedded in the overall guideline. Common recommendations are to establish safe skin-to-skin contact immediately after birth, to keep the neonate warm and to facilitate breastfeeding (50, 52, 54–57, 60, 62). In our opinion, a guideline should also aim to reduce the burden of treatment to the least possible. Therefore, less burdensome measures such as the use of dextrose gel and the short-term use of formula milk might be considered helpful in reducing the need for more invasive procedures in at-risk neonates.

Existing guidelines: who to screen?

Recommendations on which children should receive a blood glucose screening vary considerably between guidelines, ranging from four (5) to >25 risk factors (56). The only risk factor for which blood glucose screening is uniformly recommended is maternal diabetes (5, 50–57, 59, 60, 62, 63). Furthermore, there is a consensus that low birth weight (BW) infants are at increased risk for hypoglycemia, but there are different definitions for "low BW" or "SGA" depending on the guideline (e.g. BW <- 2 Standard Deviation Score (SDS) (55), BW ≤2nd centile (54), BW <10th centile (59), BW <2500 g or <3rd centile (57)). The same applies to neonates born with a "high BW" or "LGA". Definitions range from BW >90th centile (59) or BW >+2 SDS (55) to BW >4.500 g (52) etc.

The difficulty of assessing the evidence for selective screening is exemplified on the basis of the study by Brand et al. who investigated the neurodevelopmental outcome at the age of 4 years in 75 healthy term LGA infants with transient hypoglycemia, that were born to non-diabetic mothers (67). The lowest blood glucose levels observed during the first five hours was 0.6 mmol/l (11 mg/dl) and the mean was 1.9 mmol/l (34 mg/dl). Of note, twenty-seven children (36%) were treated with intravenous glucose infusion and their mean blood glucose concentration was 1.4 mmol/l (25 mg/dl). With this intensive therapeutic approach, the neurological outcome for LGA-infants with hypoglycemia was not worse than without hypoglycemia. The conclusion of the authors is that transient mild hypoglycemia in LGA neonates does not to appear harmful – although this has been shown only in a cohort in which a significant proportion received i.v. glucose. Furthermore, exclusion of LGA newborns from screening would pose a risk to neonates with congenital hyperinsulinism, who are often LGA, and thus may be underestimated and identified too late, increasing the risk of severe brain damage for this patient population (32, 33).

For some potential risk factors evidence is low. For example, for neonates with polycythemia, which is listed as a risk factor in the Queensland guideline, the Turkish guideline and by Wight et al. (51, 56, 58). Hopfeld-Fogel et al. found that neonatal hypoglycemia was not more common compared to controls with normal hematocrit (68). On the other hand, the risk factor maternal beta-blocker treatment is only listed in some guidelines (50, 51, 54, 58, 62), although a large meta-analysis recently found that the risk of hypoglycemia in neonates of mothers treated with beta-blockers can be demonstrate with moderate certainty of evidence. Accordingly, the authors recommend a postnatal glycemic monitoring during the first 24 hours (69). Further assessment of the detailed evidence for the entire set of risk factors is not possible within the scope of this review. However, it is an important topic for separate meta-analyses or systematic reviews.

Existing guidelines: blood glucose screening

No consensus exists on the optimal timing for the first postnatal blood glucose measurement. Most guidelines recommend blood glucose testing before the 2nd feed but no later than 3 to 4 hours of age (51, 52, 54, 55, 57). Other recommendations range from 1 hour of age (50) to 30 minutes after the first feed (5, 58) or the time points differ depending on the risk factor (56, 63).

Hypoglycemia thresholds for neonates below 48/72 hours of age range from <2.0 mmol/l (<36 mg/dl) to <2.6 mmol/l (<47 mg/dl). Interestingly, 10 of the 13 reported guidelines (Table 1) differentiate between symptomatic and asymptomatic hypoglycemia in their recommendations for further management. However, as mentioned above it was found that the recognition of hypoglycemia signs is very observer dependent and "that it is difficult to distinguish the nonspecific signs of normal adaptation from signs of hypoglycemia" (4). Similarly, just as glucose thresholds vary widely, the further procedures in the event of hypoglycemia, e.g., when to start intravenous glucose, varies by guideline (Table 1). Some guidelines include further potential therapeutic measures such as buccal dextrose/glucose gel (50, 51, 54–57, 59, 60, 62), a glucagon bolus (50, 52, 54, 62) or continuously administered glucagon (51). In some guidelines, the duration of screening varies according to the risk factor (5, 56, 58, 59, 63), in others, it is based on the severity of hypoglycemia (54, 60). Both seem to make sense to us. An important aspect is also the recognition of severe hypoglycemic disorders and under which conditions these must be thought of or when one can safely discharge newborns with hypoglycemia without overlooking a persistent or transient hypoglycemic disease (7). We believe it is necessary for guidelines to include recommendations such as a reasonably long "safety" fasting test before discharge in neonates with hypoglycemia persisting beyond 48/72 hours, or in neonates with suspected or confirmed hypoglycemic disease (50–52, 56, 58, 59). The evidence of "how to screen" is very low, and therefore today a pragmatic approach must be chosen that also clarifies when glucose screening should be discontinued.

Discussion, authors conclusions and implications for future research

Taken together, there is still insufficient data to define how low a glucose concentration must be, respectively, how long it must

persist at which level to cause brain injury in neonates. Furthermore, the vulnerability for adverse consequences of hypoglycemia may vary for different context factors, such as immaturity, predisposing risk factors, availability of alternative fuels etc.

Comparing different guidelines worldwide, it becomes clear that the insufficient data basis leads to considerably different interpretation and management recommendations.

Thus, further studies on these questions are needed and have been demanded by experts for years. Ideas for ideal study designs have been proposed including studies in prospective cohorts with nested randomized treatment trial or randomized trials of different approaches to prevention or screening and diagnosis of hypoglycemia in at-risk neonates (38, 70, 71).

Until data from such studies is available, the main question is how to interpret available evidence and follow the principle of "primum non nocere" in terms of prevention, screening strategy and treatment.

Should selective screening of blood glucose in at-risk newborns be adhered to and who is at risk?

Neonatal hypoglycemia screening affects a high number of neonates - depending on the definition, approximately one third of newborns have at least one risk factor for hypoglycemia. The diagnosis of hypoglycemia has relevant implications for management and care of neonates. In one recent study, 529 of 10,533 infants were admitted to the NICU postpartum, and of those, almost half (n= 235, 44,4%) for hypoglycemia management (72).

However, it has been postulated recently that screening for neonatal hypoglycemia does not meet the principles for a screening test (73). It was discussed that the screening may cause harm and that the current screening approach does not prevent severe hypoglycemia and severe brain damage.

Despite the paucity of evidence for any specific evidence-based approach, it is without doubt that there is a considerable number of children suffering from hypoglycemic brain injury because of insufficient screening and management strategies – e.g., those with inborn hypoglycemia disorders such as congenital hyperinsulinism. Furthermore, it is also without doubt that also transient hyperinsulinemic hypoglycemia without predisposing risk factors may cause hypoglycemic brain injury of varying severity (32, 34). The authors advocate that – *particularly* as there is limited evidence on timing and interventional cut-offs for screening and treatment - we owe the affected newborns balanced and thoughtful guidelines to the best of our knowledge, to be diagnosed timely and treated adequately to prevent adverse outcomes that negatively affect their lives permanently.

Preventive and screening strategies, as well as therapeutic efforts, impose only a temporary and usually minor burden. Therefore, the authors advocate accepting some degree of overtreatment to prevent long-term impairment in some - similar to the screening and treatment of neonatal hyperbilirubinemia, which implies a significant number of interventions on patients that might not suffer from negative consequences even untreated, to reliably identify and treat those who are at immediate risk for hyperbilirubinemic brain damage (74).

In newborns at risk for hypoglycemia, blood glucose should be checked according to a predefined schedule, usually starting from 2-3 hours of life. As summarized before, published recommendations vary considerably regarding the risk factors that should trigger systematic screening, and regarding the interventions. At least for the most important risk factors SGA/fetal growth restriction (FGR), maternal diabetes, preterm birth, and other forms of perinatal stress, clear recommendations for screening are needed, based on currently available data. However, predicting postnatal blood glucose concentrations is not straightforward in infants at risk (47) and it is unclear which neonates with other risk factors may need to be screened for neonatal hypoglycemia. Certainly, it seems to be justified for other risk factors, such as maternal beta-blocker therapy.

Which therapeutic measures are appropriate in which situations?

Management of at-risk newborns does not start with blood glucose screening. Preventive measures can be certainly effective without causing harm, including frequent feeding, keeping the newborn warm, and ensuring safe skin-to-skin contact – these measures should be made available to all at-risk newborns. Moderate measures to prevent or treat hypoglycemia can be supplemental formula feeding or dextrose gel application – both with very limited negative consequences. However, invasive measures such as intravenous glucose, glucagon treatment, or transfer to a NICU should be recommended thoughtfully and restrictively in a guideline, but then put into practice consistently with stepwise rapid escalation of treatment when indicated to reliably prevent severe and persistent hypoglycemia with potentially adverse consequences.

Interventions are e.g. recommended when the blood glucose concentration falls below a critical threshold and does not rise above this value, or when symptoms are observed (46). Commonly, below 2.5 mmol/l (45 mg/dl) "action" is recommended in any newborn with signs attributed to hypoglycemia (46). However, based on blood glucose levels alone, there is some debate if action should be initiated at threshold values <2.0 mmol/l or <2.5 mmol/l (<36 mg/dl or <45 mg/dl). While a single blood glucose measurement between 2.0 and 2.5 mmol/l (36 and 45 mg/dl) might not make a big difference, low values that occur repeatedly (indicating a certain severity), are accompanied by symptoms (indicating neuroglycopenia), or occur during a period of insufficient feeding (indicating limited potential for spontaneous recovery) are of

greater concern. In terms of symptoms, it should be considered that insufficient feeding despite low glucose values could indicate apathy caused by neuroglycopenia.

We recommend the operational threshold and the target blood glucose value to aim for should be >2.5 mmol/l (>45 mg/dl) from at least the 4th (-6th) hour of life (75). This higher threshold seems more appropriate to us to provide a margin of safety and to safely avoid blood glucose levels <1.7 mmol/l (<30 mg/dl). Especially prolonged periods or repeated periods in this range must be considered potentially harmful and a value that triggers invasive measures.

How are neonates with relevant hypoglycemia identified among neonates without known risk factors?

Because routine blood glucose screening is usually not performed in asymptomatic, healthy, term newborns, neonates with transitory or transient hypoglycemia as well as a permanent hypoglycemic disease, who do not have any risk factors for hypoglycemia are often first noticed by clinical signs (32). Therefore, without a general glucose screening, at least careful education and awareness of parents, midwives, and nurses regarding the clinical signs of hypoglycemia is needed. When a neonate presents with signs suggestive of hypoglycemia, e.g., adrenergic or neuroglycopenic symptoms, it is consensus that a blood glucose determination should be performed. However, given the limited sensitivity and specificity of symptoms, in case of doubt regarding signs of low glucose, only a blood glucose measurement can reliably detect or exclude hypoglycemia, and should therefore be performed quite generously ("glucose as a vital sign").

Regarding missing the early diagnosis of congenital hypoglycemic disorders or transient hyperinsulinism in the neonatal period, it is important that treatment standards define criteria for when to consider such a condition e.g., severity and/or duration of hypoglycemia, carbohydrate requirement etc. Today, these newborns often receive appropriate treatment for severe hyperinsulinism, but only after a significant delay (32). However, criteria should also be defined for the termination of preventive, screening, or therapeutic measures when there is no longer a relevant risk for recurrent or severe hypoglycemia. In unclear cases, a short diagnostic fasting test over 5-6 hours may also be helpful to exclude further hypoglycemic risk.

Future research

Severe hypoglycemic brain damage may not occur in many patients classified as having transitory hypoglycemia in the first days of life. More likely is the occurrence of minor intelligence reduction or partial performance deficits which may manifest too late in childhood to be consciously attributed to neonatal hypoglycemia (38). It can be assumed that at-risk newborns are particularly susceptible because they have, depending on the condition, low energy reserves overall, but especially in the brain. In addition, it seems likely that their limited adaptive capacities can only provide insufficient energy in the form of glucose, lactate, or ketone bodies. To prove this in studies, clinically and metabolically well characterized at-risk newborns, must be followed up neurologically in detail and on a long-term basis. Lower thresholds beyond 2.5 mmol/l (45 mg/dl) should be investigated as primary outcome to see if these are associated with worse outcomes. In particular, the duration and the frequency of hypoglycemia should be included in the analysis.

In parallel, guidelines should be prospectively evaluated in terms of efficacy regarding glycemic control and long-term neurological outcome at least until mid-childhood, when partial performance disorders become apparent. The studies should be powered to detect even small reductions in neurodevelopmental outcomes. On a population level, even small effects, e.g., a quarter of a standard deviation rather than half a standard deviation, as frequently used in studies, can be highly clinically relevant. Unnecessary overtreatment should also be avoided, and a long-term goal should be to establish evidenced-based guidelines that provide an appropriate approach for the entire neonatal population. These demands result in very challenging study designs in terms of the number of subjects and study duration. On the other hand, the topic is of such high relevance that this high effort seems justified. Authors' recommendation for future studies are summarized in Table 2.

TABLE 2 Recommendations on possible targets for future studies to increase evidence for the management of neonatal hypoglycemia.

- Prospective, preferably multicenter randomized controlled trials evaluating long-term outcome until at least mid-childhood with power to detect even small reductions in neurodevelopmental outcomes depending on:
  - different risk factors (between risk factors and within a specific risk factor group)
  - different operational thresholds (overtreatment vs. undertreatment)
  - duration and frequency of hypoglycemia
  - different preventive and treatment methods (e.g. guidelines)
  - early identification of inborn endocrine or metabolic disease causing severe neonatal hypoglycemia
  - symptomatic/asymptomatic hypoglycemia
  - the influence of alternative cerebral energy fuels (e.g. ketones, lactate)
- Prospective cohort studies evaluating the individual risk for severe hypoglycemia according to different risk factors and development of risk factor specific screening and treatment approaches
- Randomized controlled trials of signs of hypoglycemia: Does training of parents, midwives, and nurses affect early detection of severe hypoglycemia?

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Conflict of interest

The authors participated and are still involved in studies on the use of Dasiglucagon Zealand Pharma in congenital hyperinsulinism. The authors declare that the research was conducted in the absence of any other commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Whipple AO. The surgical therapy of hyperinsulinismus. *J Int Chir* (1938) 3:237–76.

2. Cryer PE, Axelrod L, Grossman AB, Heller SR, Montori VM, Seaquist ER, et al. Evaluation and management of adult hypoglycemic disorders: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2009) 94(3):709–28. doi: 10.1210/jc.2008-1410

3. International Hypoglycaemia Study Group. Glucose concentrations of less than 3.0 mmol/l (54 mg/dl) should be reported in clinical trials: a joint position statement of the American diabetes association and the European association for the study of diabetes. *Diabetologia* (2017) 60(1):3–6. doi: 10.1007/s00125-016-4146-6

4. Hoermann H, Mokwa A, Roeper M, Salimi Dafsari R, Koestner F, Hagenbeck C, et al. Reliability and observer dependence of signs of neonatal hypoglycemia. *J Pediatr* (2022) 245:22–9.e2. doi: 10.1016/j.jpeds.2022.02.045

5. Committee on Fetus and Newborn, Adamkin DH. Postnatal glucose homeostasis in late-preterm and term infants. *Pediatrics* (2011) 127(3):575–9. doi: 10.1542/peds.2010-3851

6. World Health Organization. *Hypoglycaemia of the newborn: review of the literature*. World Health Organization (1997). Available at: https://apps.who.int/iris/handle/10665/63362 (Accessed May 25, 2023).

7. 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

8. Hartmann AF, Jaudon JC, Morton M. Hypoglycemia. *J pediatrics* (1937) 11:1–36.

9. Stanley CA, Anday EK, Baker L, Delivoria-Papadopolous M. Metabolic fuel and hormone responses to fasting in newborn infants. *Pediatrics* (1979) 64(5):613–9.

10. Wright LL, Stanley CA, Anday EK, Baker L. The effect of early feeding on plasma glucose levels in SGA infants. *Clin Pediatr (Phila)* (1983) 22(8):539–41. doi: 10.1177/000992288302200803

11. Harris DL, Gamble GD, Weston PJ, Harding JE. What happens to blood glucose concentrations after oral treatment for neonatal hypoglycemia? *J Pediatr* (2017) 190:136–41. doi: 10.1016/j.jpeds.2017.06.034

12. Srinivasan G, Pildes RS, Cattamanchi G, Voora S, Lilien LD. Plasma glucose values in normal neonates: a new look. *J Pediatr* (1986) 109(1):114–7. doi: 10.1016/s0022-3476(86)80588-1

13. Diwakar KK, Sasidhar MV. Plasma glucose levels in term infants who are appropriate size for gestation and exclusively breast fed. *Arch Dis Child Fetal Neonatal Ed* (2002) 87(1):F46–8. doi: 10.1136/fn.87.1.f46

14. Alkalay AL, Sarnat HB, Flores-Sarnat L, Elashoff JD, Farber SJ, Simmons CF. Population meta-analysis of low plasma glucose thresholds in full-term normal newborns. *Am J Perinatol* (2006) 23(2):115–9. doi: 10.1055/s-2006-931912

15. Harris DL, Weston PJ, Gamble GD, Harding JE. Glucose profiles in healthy term infants in the first 5 days: the glucose in well babies (GLOW) study. *J Pediatr* (2020) 223:34–41.e4. doi: 10.1016/j.jpeds.2020.02.079

16. Harris DL, Weston PJ, Harding JE. Lactate, rather than ketones, may provide alternative cerebral fuel in hypoglycaemic newborns. *Arch Dis Child Fetal Neonatal Ed* (2015) 100(2):F161–4. doi: 10.1136/archdischild-2014-306435

17. Falkowska A, Gutowska I, Goschorska M, Nowacki P, Chlubek D, Baranowska-Bosiacka I. Energy metabolism of the brain, including the cooperation between astrocytes and neurons, especially in the context of glycogen metabolism. *Int J Mol Sci* (2015) 16(11):25959–81. doi: 10.3390/ijms161125939

18. Singh M, Singhal PK, Paul VK, Deorari AK, Sundaram KR, Ghorpade MD, et al. Neurodevelopmental outcome of asymptomatic & symptomatic babies with neonatal hypoglycaemia. *Indian J Med Res* (1991) 94:6–10.

19. Koivisto M, Blanco-Sequeiros M, Krause U. Neonatal symptomatic and asymptomatic hypoglycaemia: a follow-up study of 151 children. *Dev Med Child Neurol* (1972) 14(5):603–14. doi: 10.1111/j.1469-8749.1972.tb02642.x

20. Koh TH, Aynsley-Green A, Tarbit M, Eyre JA. Neural dysfunction during hypoglycaemia. *Arch Dis Child* (1988) 63(11):1353–8. doi: 10.1136/adc.63.11.1353

21. Alkalay AL, Flores-Sarnat L, Sarnat HB, Moser FG, Simmons CF. Brain imaging findings in neonatal hypoglycemia: case report and review of 23 cases. *Clin Pediatr (Phila)* (2005) 44(9):783–90. doi: 10.1177/000992280504400906

22. Burns CM, Rutherford MA, Boardman JP, Cowan FM. Patterns of cerebral injury and neurodevelopmental outcomes after symptomatic neonatal hypoglycemia. *Pediatrics* (2008) 122(1):65–74. doi: 10.1542/peds.2007-2822

23. Barkovich AJ, Ali FA, Rowley HA, Bass N. Imaging patterns of neonatal hypoglycemia. *AJNR Am J Neuroradiol* (1998) 19(3):523–8.

24. Murakami Y, Yamashita Y, Matsuishi T, Utsunomiya H, Okudera T, Hashimoto T. Cranial MRI of neurologically impaired children suffering from neonatal hypoglycaemia. *Pediatr Radiol* (1999) 29(1):23–7. doi: 10.1007/s002470050527

25. Filan PM, Inder TE, Cameron FJ, Kean MJ, Hunt RW. Neonatal hypoglycemia and occipital cerebral injury. *J Pediatr* (2006) 148(4):552–5. doi: 10.1016/j.jpeds.2005.11.015

26. Yalnizoglu D, Haliloglu G, Turanli G, Cila A, Topcu M. Neurologic outcome in patients with MRI pattern of damage typical for neonatal hypoglycemia. *Brain Dev* (2007) 29(5):285–92. doi: 10.1016/j.braindev.2006.09.011

27. Mao J, Chen LY, Fu JH, Li J, Xue XD. Clinical evaluation by MRI on the newborn infants with hypoglycemic brain damage. *Zhonghua Er Ke Za Zhi.* (2007) 45(7):518–22.

28. Tam EW, Widjaja E, Blaser SI, Macgregor DL, Satodia P, Moore AM. Occipital lobe injury and cortical visual outcomes after neonatal hypoglycemia. *Pediatrics* (2008) 122(3):507–12. doi: 10.1542/peds.2007-2002

29. Kim SY, Goo HW, Lim KH, Kim ST, Kim KS. Neonatal hypoglycaemic encephalopathy: diffusion-weighted imaging and proton MR spectroscopy. *Pediatr Radiol* (2006) 36(2):144–8. doi: 10.1007/s00247-005-0020-2

30. Traill Z, Squier M, Anslow P. Brain imaging in neonatal hypoglycaemia. *Arch Dis Child Fetal Neonatal Ed* (1998) 79(2):F145–7. doi: 10.1136/fn.79.2.f145

31. Nivins S, Kennedy E, Thompson B, Gamble GD, Alsweiler JM, Metcalfe R, et al. Associations between neonatal hypoglycaemia and brain volumes, cortical thickness and white matter microstructure in mid-childhood: an MRI study. *NeuroImage Clin* (2022) 33:102943. doi: 10.1016/j.nicl.2022.102943

32. Roeper M, Salimi Dafsari R, Hoermann H, Mayatepek E, Kummer S, Meissner T. Risk factors for adverse neurodevelopment in transient or persistent congenital hyperinsulinism. *Front Endocrinol (Lausanne)* (2020) 11:580642. doi: 10.3389/fendo.2020.580642

33. 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

34. 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

35. 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(11):4133–9. doi: 10.1210/jc.2015-2539

36. 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

37. Lucas A, Morley R, Cole TJ. Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. *BMJ* (1988) 297(6659):1304–8. doi: 10.1136/bmj.297.6659.1304

38. Shah R, Harding J, Brown J, McKinlay C. Neonatal glycaemia and neurodevelopmental outcomes: a systematic review and meta-analysis. *Neonatology* (2019) 115(2):116–26. doi: 10.1159/000492859

39. Harris DL, Alsweiler JM, Ansell JM, Gamble GD, Thompson B, Wouldes TA, et al. Outcome at 2 years after dextrose gel treatment for neonatal hypoglycemia: follow-up of a randomized trial. *J Pediatr* (2016) 170:54–9.e1-2. doi: 10.1016/j.jpeds.2015.10.066

40. McKinlay CJD, Alsweiler JM, Anstice NS, Burakevych N, Chakraborty A, Chase JG, et al. Association of neonatal glycemia with neurodevelopmental outcomes at 4.5 years. *JAMA Pediatr* (2017) 171(10):972–83. doi: 10.1001/jamapediatrics.2017.1579

41. Shah R, Dai DWT, Alsweiler JM, Brown GTL, Chase JG, Gamble GD, et al. Association of neonatal hypoglycemia with academic performance in mid-childhood. *JAMA* (2022) 327(12):1158–70. doi: 10.1001/jama.2022.0992

42. Rasmussen AH, Wehberg S, Portner F, Larsen AM, Filipsen K, Christesen HT. Neurodevelopmental outcomes after moderate to severe neonatal hypoglycemia. *Eur J Pediatr* (2020) 179(12):1981–91. doi: 10.1007/s00431-020-03729-x

43. van Kempen A, Eskes PF, Nuytemans D, van der Lee JH, Dijksman LM, van Veenendaal NR, et al. Lower versus traditional treatment threshold for neonatal hypoglycemia. *N Engl J Med* (2020) 382(6):534–44. doi: 10.1056/NEJMoa1905593

44. Kaiser JR, Bai S, Gibson N, Holland G, Lin TM, Swearingen CJ, et al. Association between transient newborn hypoglycemia and fourth-grade achievement test proficiency: a population-based study. *JAMA Pediatr* (2015) 169(10):913–21. doi: 10.1001/jamapediatrics.2015.1631

45. Wickstrom R, Skiold B, Petersson G, Stephansson O, Altman M. Moderate neonatal hypoglycemia and adverse neurological development at 2-6 years of age. *Eur J Epidemiol* (2018) 33(10):1011–20. doi: 10.1007/s10654-018-0425-5

46. 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

47. Rozance PJ, Hay WWJr. Describing hypoglycemia–definition or operational threshold? *Early Hum Dev* (2010) 86(5):275–80. doi: 10.1016/j.earlhumdev.2010.05.002

48. Koh TH, Vong SK. Definition of neonatal hypoglycaemia: is there a change? *J Paediatr Child Health* (1996) 32(4):302–5. doi: 10.1111/j.1440-1754.1996.tb02558.x

49. Roeper M, Salimi Dafsari R, Hoermann H, Hoehn T, Kummer S, Meissner T. Clinical management and knowledge of neonatal hypoglycaemia in Germany: a national survey of midwives and nurses. *J Paediatr Child Health* (2021) 57(6):854–9. doi: 10.1111/jpc.15337

50. Varghese J, et al. South Australian perinatal practice guideline, in: *Neonatal hypoglycaemia: department for health and wellbeing* (2020). Government of South Australia. Available at: https://www.sahealth.sa.gov.au/ (Accessed March 1, 2023).

51. Queensland Clinical Guidelines. *Hypoglycaemia-newborn. guideline no. MN19.8-V12-R24: Queensland health* (2022). Available at: http://www.health.qld.gov.au/ (Accessed March 1, 2023).

52. Government of Western Australia. *Child and adolescent health service. hypoglycaemia guideline* (2021). Available at: https://www.cahs.health.wa.gov.au/ (Accessed March 1, 2023).

53. Segerer H, Bührer C, Kapellen T, Mattern E, Ramsauer B, Somville T, et al. Betreuung von neugeborenen diabetischer mütter. leitlinie der GNPI, DGPM, DDG, DGHWi, dem DHV, der DGKJ und DGGG.(2k-level, AWMF-Leitlinien-Register nr. 024/006, juli 2017). *Z für Geburtshilfe und Neonatologie* (2018) 222(03):107–14. doi: 10.1055/a-0621-1369

54. British Association of Perinatal Medicine. *Identification and management of neonatal hypoglycemia in the full term infant framework for practice* (2017). Available at: https://www.bapm.org/ (Accessed March 1, 2023).

55. Wackernagel D, Gustafsson A, Edstedt Bonamy AK, Reims A, Ahlsson F, Elfving M, et al. Swedish National guideline for prevention and treatment of neonatal hypoglycaemia in newborn infants with gestational age >/=35 weeks. *Acta Paediatr* (2020) 109(1):31–44. doi: 10.1111/apa.14955

56. Wight NEAcademy of Breastfeeding M. ABM clinical protocol #1: guidelines for glucose monitoring and treatment of hypoglycemia in term and late preterm neonates, revised 2021. *Breastfeed Med* (2021) 16(5):353–65. doi: 10.1089/bfm.2021.29178.new

57. Working Group of the Swiss Society of Neonatology (SSN). *Prevention and treatment of hypoglycaemia in neonates with a gestational age from 35 0/7 weeks in maternity wards: Swiss society of neonatology guidelines* (2020). Available at: https://www.neonet.ch/ (Accessed March 1, 2023).

58. Aliefendioglu D, Coban A, Hatipoglu N, Ecevit A, Arisoy AE, Yesiltepe G, et al. Management of hypoglycemia in newborn: Turkish neonatal and pediatric endocrinology and diabetes societies consensus report. *Turk Pediatri Ars* (2018) 53 (Suppl 1):S224–S33. doi: 10.5152/TurkPediatriArs.2018.01820

59. Narvey MR, Marks SD. The screening and management of newborns at risk for low blood glucose. *Paediatr Child Health* (2019) 24(8):536–54. doi: 10.1093/pch/pxz134

60. Maternity Guidelines Group. *Christchurch Women's hospital. hypoglycaemia of the newborn* (2020). Available at: https://edu.cdhb.health.nz/ (Accessed March 1, 2023).

61. Unicef Uk Baby Friendly Initiative. *Guidance on the development of policies and guidelines for the prevention and management of hypoglycaemia of the newborn: UNICEF London* (2013). Available at: www.babyfriendly.org.uk (Accessed March 1, 2023).

62. Waitemata District Health Board. *Neonatal hypoglycaemia guideline* (2021). Available at: www.healthpoint.co.nz (Accessed March 1, 2023).

63. Rasmussen AH, Wehberg S, Fenger-Groen J, Christesen HT. Retrospective evaluation of a national guideline to prevent neonatal hypoglycemia. *Pediatr Neonatol* (2017) 58(5):398–405. doi: 10.1016/j.pedneo.2016.12.002

64. Gupta K, Amboiram P, Balakrishnan U, A C, Abiramalatha T, Devi U. Dextrose gel for neonates at risk with asymptomatic hypoglycemia: a randomized clinical trial. *Pediatrics* (2022) 149(6):e2021050733. doi: 10.1542/peds.2021-050733

65. Parappil H, Gaffari M, Ahmed J, Skaria S, Rijims M, Chandra P, et al. Oral dextrose gel use in asymptomatic hypoglycemic newborns decreases NICU admissions and parenteral dextrose therapy: a retrospective study. *J Neonatal Perinatal Med* (2023) 16(1):111–7. doi: 10.3233/NPM-221170

66. Meneghin F, Manzalini M, Acunzo M, Daniele I, Bastrenta P, Castoldi F, et al. Management of asymptomatic hypoglycemia with 40% oral dextrose gel in near term at-risk infants to reduce intensive care need and promote breastfeeding. *Ital J Pediatr* (2021) 47(1):201. doi: 10.1186/s13052-021-01149-7

67. Brand PL, Molenaar NL, Kaaijk C, Wierenga WS. Neurodevelopmental outcome of hypoglycaemia in healthy, large for gestational age, term newborns. *Arch Dis Child* (2005) 90(1):78–81. doi: 10.1136/adc.2003.039412

68. Hopfeld-Fogel A, Kasirer Y, Mimouni FB, Hammerman C, Bin-Nun A. Neonatal polycythemia and hypoglycemia in newborns: are they related? *Am J Perinatol* (2021) 38(9):930–4. doi: 10.1055/s-0040-1701193

69. de Bruin R, van Dalen SL, Franx SJ, Ramaswamy VV, Simons SHP, Flint RB, et al. The risk for neonatal hypoglycemia and bradycardia after beta-blocker use during pregnancy or lactation: a systematic review and meta-analysis. *Int J Environ Res Public Health* (2022) 19(15):9616. doi: 10.3390/ijerph19159616

70. Boluyt N, van Kempen A, Offringa M. Neurodevelopment after neonatal hypoglycemia: a systematic review and design of an optimal future study. *Pediatrics* (2006) 117(6):2231–43. doi: 10.1542/peds.2005-1919

71. Rozance PJ. Hypoglycemia in the newborn and neurodevelopmental outcomes in childhood. *JAMA* (2022) 327(12):1135–7. doi: 10.1001/jama.2022.2456

72. Mukhopadhyay S, Wade KC, Dhudasia MB, Skerritt L, Chou JH, Dukhovny D, et al. Clinical impact of neonatal hypoglycemia screening in the well-baby care. *J Perinatol* (2020) 40(9):1331–8. doi: 10.1038/s41372-020-0641-1

73. Alsweiler JM, Heather N, Harris DL, McKinlay CJD. Application of the screening test principles to screening for neonatal hypoglycemia. *Front Pediatr* (2022) 10:1048897. doi: 10.3389/fped.2022.1048897

74. Lai NM, Gerard JP, Ngim CF, Kamar AA, Chen KH. The association between serum bilirubin and kernicterus spectrum disorder: a systematic review and meta-analysis. *Neonatology* (2021) 118(6):654–64. doi: 10.1159/000519497

75. Hoermann H, Roeper M, Dafsari RS, Koestner F, Schneble D, von Zezschwitz D, et al. Protecting against brain damage by improving treatment in neonates with hypoglycaemia: ProBrain-d-a study protocol of a prospective longitudinal study. *BMJ Open* (2022) 12(8):e063009. doi: 10.1136/bmjopen-2022-063009

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Watchful waiting versus pharmacological management of small-for-gestational-age infants with hyperinsulinemic hypoglycemia

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Introduction: Given that reports on severe diazoxide (DZX) toxicity are increasing, we aimed to understand if the short-term clinical outcomes of small-for-gestational-age (SGA) infants with hyperinsulinemic hypoglycemia (HH) managed primarily by supportive care, termed watchful waiting (WW), are different from those treated with DZX.

Method: A real-life observational cohort study was conducted from 1 September 2014 to 30 September 2020. The WW or DZX management decision was based on clinical and biochemical criteria. We compared central line duration (CLD), postnatal length of stay (LOS), and total intervention days (TIDs) among SGA-HH infants treated with DZX versus those on a WW approach. Fasting studies determined the resolution of HH.

Result: Among 71,836 live births, 11,493 were SGA, and 51 SGA infants had HH. There were 26 and 25 SGA-HH infants in the DZX and WW groups, respectively. Clinical and biochemical parameters were similar between groups. The median day of DZX initiation was day 10 of life (range 4–32), at a median dose of 4 mg/kg/day (range 3–10). All infants underwent fasting studies. Median CLD [DZX, 15 days (6–27) vs. WW, 14 days (5–31), P = 0.582] and postnatal LOS [DZX, 23 days (11–49) vs. WW, 22 days (8–61), P = 0.915] were comparable. Median TID was >3-fold longer in the DZX than the WW group [62.5 days (9–198) vs. 16 days (6–27), P < 0.001].

Conclusion: CLD and LOS are comparable between WW and DZX groups. Since fasting studies determine the resolution of HH, physicians should be aware that clinical intervention of DZX-treated SGA-HH patients extends beyond the initial LOS.

KEYWORDS

diazoxide, hyperinsulinemic hypoglycemia, fasting study, small-for-gestational-age, watchful waiting

Introduction

Many small-for-gestational-age (SGA) infants are substrate deficient and, therefore, at risk of hypoglycemia (1). Since the likelihood of developing hypoglycemia is greatest in the first hours of life when the physiological glucose nadir occurs, SGA babies need to be fed soon after birth and monitored (2). Other mechanisms of hypoglycemia include hyperinsulinemic hypoglycemia (HH), impaired gluconeogenesis/glycogenolysis, and adrenocortical insufficiency (3, 4). Approximately 1 in 200 SGA infants will develop HH during neonatal life, primarily attributed to perinatal stress hyperinsulinism (PSHI), which is usually transient (5, 6). Yet, at initial presentation, it is clinically difficult to predict if the temporal nature of HH will be transient, prolonged, or persistent and whether the etiology will be genetic or not.

Clinicians managing SGA-HH infants are challenged in two ways. The first question is whether HH will be prolonged or persistent since PSHI has been reported to continue from 18 to 403 days or if it will be transient and spontaneously resolve within a week (7, 8). The second challenge relates to pharmacotherapy, as it is difficult to predict the SGA-HH infant who will benefit from diazoxide (DZX) therapy while at the same time identifying those at the highest risk of developing side effects (5). Clinicians who favor pharmacotherapy are often persuaded by the economic consequences of prolonged LOS and the fear of litigation (9). On the other hand, the growing list of published side effects of DZX therapy warrants caution (10, 11). Furthermore, whether SGA-HH babies treated with DZX fare better than those managed more conservatively without pharmacotherapy remains fundamentally unclear.

We hypothesized that the short-term outcomes of SGA-HH infants treated with DZX are not better than those managed by watchful waiting (WW). The primary aim of our study was to compare the central venous line duration (CLD), length of stay (LOS), and total intervention days (TIDs) till resolution of HH among watchful waiting (WW group) versus DZX-treated (DZX group) SGA infants with HH.

Materials and methods

Study setting and patient population

This observational cohort study was conducted in a real-life setting over 73 months from 1 September 2014 to 30 September 2020 at KK Women's and Children's Hospital Singapore, a national tertiary referral center with an annual birth rate of approximately 11,000 per year. All SGA infants with HH during the study period were included. We included infants in the intensive care ward who were admitted primarily for central line HH management and had no other major medical or surgical conditions. We excluded infants who did not have a fasting study that demonstrated resolution (Figure 1). This study was granted exemption from ethics approval by the Centralized Institutional Review Board (CIRB 2021/2166).

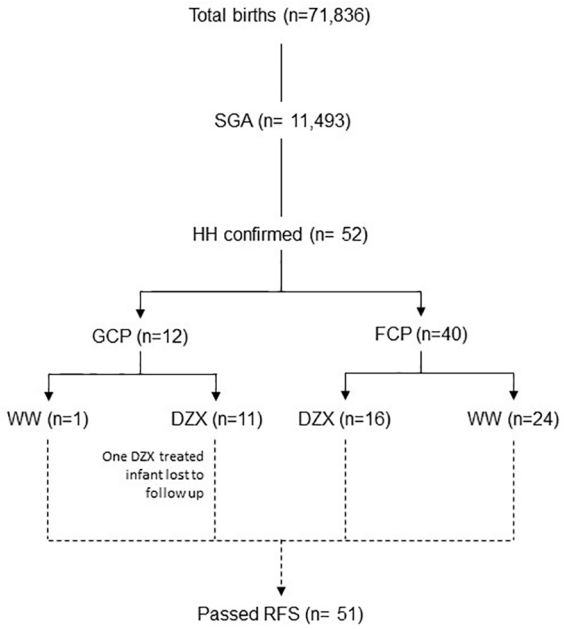

FIGURE 1
The flow diagram of participants included in the study. DZX, diazoxide; HH, hyperinsulinemic hypoglycemia; FCP; feed-centric pathway; GCP, glucose-centric pathway; RFS, resolution fasting study; SGA, small-for-gestational-age; WW, watchful waiting.

Definitions

We defined SGA as those with a birth weight of <10th centile for gestational age using the Fenton reference (12). We defined hypoglycemia according to the pathway in use (glucose-centric or feed-centric pathway), as detailed below. We confirmed HH in any infant >48 h of life with (a) hypoglycemia (<3.0 mmol/L), (b) inappropriate insulin levels (≥ 1.6 mU/L (chemiluminescence microparticle immunoassay, Abbott, USA), (c) hypoketonemia (<0.6 mmol/L), and (d) hypofattyacidemia (<0.5 mmol/L) (13). We defined the resolution of HH by fasting studies (14, 15).

Hypoglycemia screening

Glucose screening was performed using a bedside glucometer (Abbott Optium Neo, UK). Laboratory determination of plasma glucose is performed when the glucometer reading is <3 mmol/L. All critical blood samples had paired plasma glucose with serum insulin.

Two hypoglycemia screening methods were used during the study period. The first method was a glucose-centric pathway (GCP), where blood glucose soon after birth directed the subsequent management actions. In a GCP, hypoglycemia was defined as ≤2.5 mmol/L. The GCP period covered 17 months, from 1 September 2014 to 31 January 2016. The second method was a feed-centric pathway (FCP), which instead focused on early feeding, skin-to-skin care, and glucose testing at 2 h of life. In an FCP, hypoglycemia was defined as <3 mmol/L. The FCP period

covered the remaining 56 months of the study period from 1 February 2016 to 30 September 2020. The key features of the GCP and FCP are presented in Table 1, details of which were previously published (16).

Hypoglycemia management

The approach to hypoglycemia management was pathway-specific, as described in Table 1. The principal feature in common between the GCP and FCP was the focus on providing substrate in the first 48 h and testing for HH only after 48 h in those with the glucose infusion rate (GIR) >10 mg/kg/min. The maximum GIR was recorded in our cohort before DZX initiation. In contrast to the GCP, the unique feature of the FCP was the starting point of care that included a universal skin-to-skin approach, breastfeeding first, followed by buccal glucose [40% glucose gel; dose 200 mg/kg (0.5 ml/kg)] up to two doses given 30 min apart) when required, and restricting the use of intravenous (IV) dextrose as far as possible (16). In the FCP, the use of IV glucagon was avoided, and IV hydrocortisone was used only when adrenal insufficiency was proven.

Diazoxide

The decision to commence DZX was based on clinical and biochemical criteria throughout the study. The criteria to consider DZX therapy was previously published (5).

(a) Recurrent need to increase GIR to maintain normoglycemia after 48 h of age.

(b) Recurrent episodes of low blood glucose despite increasing feeds while weaning IV glucose.

(c) Inability to achieve full feeds by 1–2 weeks of age while attempting to reduce IV glucose.

Normal full blood counts, liver and renal function parameters, and absence of pericardial effusion and pulmonary hypertension in an echocardiogram were prerequisites to start DZX. We started DZX at a dose of 3 mg/kg/day in two divided doses and increased at 2–2.5 mg/kg/day if the initial dose response was inadequate. As we have previously published, a lower starting dose was chosen (compared to the conventional dose of 5–20 mg/kg/day) based on its efficacy in treating HH in SGA infants with minimal adverse effects (5). All infants were provided with oral hydrochlorothiazide at a dose of 1 mg/kg/day in two divided doses to mitigate the fluid-retaining side effect of DZX. Once adequate response to DZX was obtained, as defined by normal pre-feed glucose for 48 h on full oral feeds, we performed a 6-h safety fasting study (SFS) before discharging home. An infant is considered to pass the fasting study if glucose levels are normal during the fasting period and at the end of the study. The SFS was designed to confirm the infant's ability to maintain glucose levels during an inadvertent fast at home. Home glucose monitoring was continued until the dose of DZX was passively weaned (with weight gain) to half the initial dose or <1.5 mg/kg/day. An active weaning of DZX was done if glucose levels were above >7 mmol/L. Infants on home glucose monitoring were tasked to inform the nurse of any hypoglycemia episodes. Subsequently, to allow clearance of DZX from the circulation, medications were discontinued for 3 days while closely monitoring symptoms and glucose levels at home. On day 4 of DZX discontinuation, the infant was admitted to the hospital for a supervised age-appropriate resolution fasting study (RFS) (5). Passing RFS qualified the infant to remain off DZX and continue with growth and neurodevelopmental follow-up. None of the DZX-treated participants required readmission for hypoglycemia after RFS.

Watchful waiting

Infants who did not receive DZX therapy were on a WW approach. We performed a 6-h fasting study before discharge once WW infants were taken off parenteral glucose and had stable pre-feed glucose for 48 h while on full oral feeds. Passing this fasting study qualified the infant safe for discharge home with

TABLE 1 Common and unique features of the glucose-centric pathway (GCP) and feed-centric pathway (FCP).

	GCP	FCP
Common features	1. Provide feeds in the first 48 h of life 2. Monitor blood glucose in the first 24 h of life in all at-risk infants 3. Provide IV dextrose for symptomatic infants 4. Critical sampling after 48 h of life 5. Critical blood sampling threshold is GIR >10mg/kg/min	
Unique features		
Target blood glucose (mmol/L)	≤2.5	<3
Time of glucose testing (hour of life)	0, 1, 3, 6, 12, 24	2, 6, 12, 18, 24, 36
Approach to intervention for asymptomatic infants	Step 1: IV dextrose/feeds Step 2: IV glucagon Step 3: IV hydrocortisone Step 4: Consider diazoxide	Step 1: Skin-to-skin and breastfeeding Step 2: Donor or formula milk Step 3: Buccal glucose gel Step 4: IV dextrose Step 5: Consider diazoxide

parental education, feeding training, observation for hypoglycemia symptoms, and safe use of buccal glucose. This fasting study was considered RFS for WW infants and defined the resolution of HH and the presence of an intact hormone regulation mechanism to maintain normoglycemia. None of the WW participants required readmission for hypoglycemia after RFS.

Outcome measures

Data collected on infants included gender, gestational age at birth, birth weight, age at presentation, symptoms, critical investigations (plasma glucose and paired glucose and insulin levels), and highest GIR. Information related to DZX included the day of initiation, the highest dose administered, and the duration of treatment. CLD, LOS, and TID were recorded as outcome measures. CLD was the interval between the insertion of the umbilical or peripherally inserted central line and its removal. LOS was the duration from birth to the day of discharge. TID was the time interval from the day of diagnosis of HH to the day when the patient passed the RFS.

Statistical analysis

We performed statistical analysis in SAS software version 9.4 for Windows (Cary, NC: SAS Institute Inc.). Demographics and clinical features were reported as descriptive statistics via frequency and percentage for categorical variables and mean ± standard deviation and median (minimum–maximum) for continuous variables. The two groups (DZX and WW) were compared using Fisher's exact test and the Mann–Whitney U test for categorical and continuous variables. The Mann–Whitney U test was also conducted to compare DZX versus WW. Due to small sample sizes per group, the normality assumption may not be tenable and non-parametric methods are used. Exploratory analysis was performed to adjust for the time of diagnosis (GCP and FCP) using a multivariable generalized linear model for the clinical outcomes, and the adjusted p values were calculated. Statistical significance was set at $p < 0.05$.

Results

Figure 2 depicts the timeline when SGA infants were consecutively diagnosed with HH during the study period and categorized by whether they received DZX or were managed by WW. Excluding one infant in the DZX group who returned overseas, there were 51 SGA infants with HH among 11,493 with SGA from 71,836 live births, giving an overall SGA-HH incidence of 1 in 225. The incidence of SGA-HH was similar in the GCP (1 in 228) and FCP (1 in 219) groups.

Table 2 describes the clinical characteristics of the 51 SGA infants with HH, of which 26 received DZX treatment and 25 had WW. Sex, gestational age, birth weight, day of presentation, plasma glucose level at presentation, day of diagnosis of HH, and paired glucose/insulin level were not different between DZX and WW infants. In the DZX group, more infants had symptoms, and their maximum GIR was higher. However, these were not statistically significant. All infants in the DZX group received DZX after confirmation of HH.

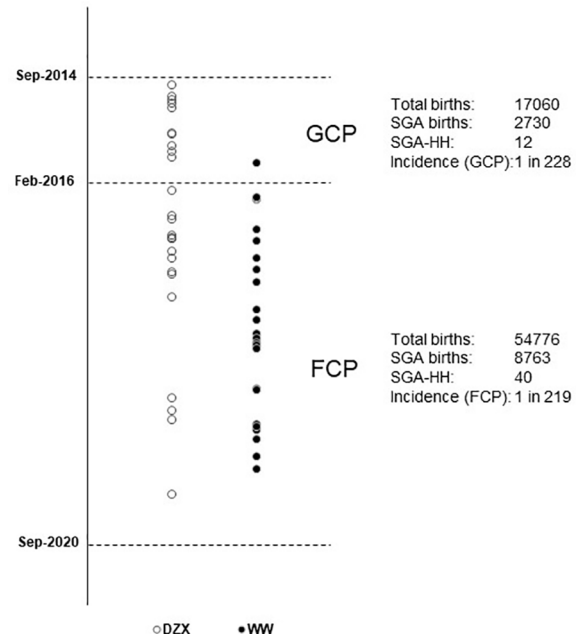

FIGURE 2
Vertical timeline of SGA infants diagnosed with HH during the study period, managed with DZX (n = 26, open o circles) and WW (n = 25, bold • circles). The respective incidence of SGA-HH during the glucose-centric pathway (GCP) and the feed-centric pathway (FCP) period is shown. DZX, diazoxide; HH, hyperinsulinemic hypoglycemia; SGA, small-for-gestational-age; WW, watchful waiting.

The median dose (mg/kg/day) of DZX received by infants in the DZX group was 4 (range 3–10). All infants in the DZX group underwent SFS, and all had RFS. In the WW group, all had documentation of the resolution of HH by RFS.

Table 3 demonstrates the clinical outcomes. The median day of DZX initiation was day 10 of life (range 4–32). Median CLD (DZX 15 days, range 6–27 vs. WW 14 days, range 5–31, P = 0.582) and postnatal LOS (DZX 23 days, range 11–49 vs. WW 22 days, range 8–61, P = 0.915) were statistically comparable between the groups. However, the median TID was 16 days (range 6–27) in the WW group, in contrast to 62.5 days (range 9–198) ($P < 0.001$) in the DZX group (Table 3). The results of the exploratory multivariable model adjusting for the time of diagnosis are comparable with the above findings (CLD-adjusted P value = 0.268; LOS adjusted P value = 0.972; TID-adjusted P value < 0.001).

Two infants (7.4%) treated with DZX 10 mg/kg/day developed marked generalized hypertrichosis, and another infant (3.7%) on DZX 4.8 mg/kg/day developed hyponatremia with pleural effusion.

For a comparison of the clinical outcomes between infants in the GCP (n = 11) and FCP (n = 40), please refer to Supplementary Table 1.

Discussion

In support of our hypothesis, our results demonstrate that the inclusion of DZX in the management of SGA infants with HH did not significantly reduce nor extend the duration of central venous

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TABLE 2 Clinical characteristics of hyperinsulinemic hypoglycemia infants treated with DZX versus WW.

Characteristic	DZX group (n = 26)	WW group (n = 25)	P value*
Sex (male)	15 (60%) †	14 (53.9%) †	0.779
Gestational age (weeks)	36.5 ± 2.1 37 (31–40)	36.1 ± 2.4 36 (30–39)	0.688
Birthweight (g)	1,948 ± 362 2,025 (1,120–2,370)	1,932 ± 500 1,915 (900–2,655)	0.985
Symptom(s) at presentation	Jittery 5 (19.2%) Seizure 1 (3.9%)	Jittery 2 (8%) Seizure 0 (0%)	0.330
Day of presentation	1.1 ± 0.4 1 (1–3)	1.0 ± 0.2 1.0 (1–2)	1.000
Day of diagnosis of HH	6.6 ± 3.3 6 (3–18)	6.2 ± 2.0 6 (3–12)	0.954
Plasma glucose (mmol/L)	1.3 ± 0.7 1.4 (0.3–2.9)	1.3 ± 0.6 1.2 (0.3–2.6)	0.828
Paired glucose/insulin levels (mmol/L)/(mU/L) #	2.4 ± 0.4/11.7 ± 12.8 2.6 (1.4–2.9)/4.6 (1.9–53)	2.2 ± 0.4/9 ± 11.2 2.3 (1.2–2.9)/6.9 (1.6–57.6)	0.051/0.734
Maximum GIR (mg/kg/min)	15.2 ± 4.2 14.4 (10–26.2)	13.7 ± 2.8 13 (10–20.3)	0.187

†Numeric variables are reported as mean ± standard deviation and median (minimum–maximum); categorical variables are reported as frequency (percent).
*The DZX and WW groups were compared using Fisher's exact test and the Mann–Whitney U test for categorical and continuous variables, respectively.
#Paired glucose/insulin levels measured after 48 h of life as part of critical samples to diagnose hyperinsulinism DZX, diazoxide; GIR, glucose infusion rate; HH, hyperinsulinemic hypoglycemia; WW, watchful waiting.

TABLE 3 Clinical outcomes of infants treated with DZX versus WW.

Variables	DZX group (n = 26)	WW group (n = 25)	P value*	Adjusted P value**
Central line duration (days)	15.5 ± 5.6 15 (6–27)	14.8 ± 6.7 14 (5–31)	0.582	0.268
Length of stay (days)	23.2 ± 9.7 23 (11–49)	24.3 ± 13 22 (8–61)	0.915	0.972
Diazoxide initiation (day of life)	13.3 ± 8.1 10 (4–32)	N.A.	N.A.	N.A.
Dose of DZX (mg/kg/day)	4.6 ± 2.1 4 (3–10)	N.A.	N.A.	N.A.
Total intervention days (days)	68 ± 40.6 62.5 (9–198)	16.6 ± 5.9 16 (6–27)	<0.001	<0.001

†Figures reported as mean ± standard deviation and median (minimum – maximum).
*Mann–Whitney U test.
**Multivariable generalized linear model adjusting for time of diagnosis.
DZX, diazoxide; WW, watchful waiting.

line placement nor shorten the postnatal LOS, compared to WW. Instead, when defined by fasting studies, DZX use substantially increased the TID by threefold among treated infants compared to those who did not receive the pharmacological intervention. This is because the methodology of DZX cessation typically involves gradual dose reduction with weight gain before cessation (5, 15). We also showed that among non-DZX treated SGA infants treated by WW in our cohort, HH resolved by a median of 16 days and not later than 27 days. We are unaware of a study that demonstrates the duration of SGA-HH in non-pharmacologically treated infants.

Our findings provide pragmatic evidence to medical practitioners that a supportive WW approach does not prolong central venous line requirement nor lengthen the duration of hospital stay compared to DZX- treated infants, also implying that DZX treatment does not shorten these parameters, which contrasts with the findings and conclusions of Balachandran et al. (17). These findings also provide clinicians, who are often faced with clinical pressure to initiate DZX therapy, with real-life clinical data that an alternative management modality is available that spares patients from the potential risks of DZX therapy including necrotizing enterocolitis (10, 11, 18), pericardial effusion (19), and pulmonary hypertension (20, 21). Despite several reports of NEC in DZX-exposed preterm and SGA infants, Gray et al. suggest that it is an association rather than causation (22). The biological

mechanism of these side effects may be due to the persistent hyperpolarization of neuronal and intestinal cells since DZX is a K_{ATP} channel agonist (10, 23).

It is established that SGA infants are at risk of impaired cognitive and neurological outcomes, whether they develop hypoglycemia or not (24, 25). Therefore, it is understandable that physicians have litigation concerns (9) and face pressure to provide active treatment when SGA infants develop low blood glucose since it may worsen outcomes and lead to neuronal injuries as a result of potential extensive white matter changes in the brain (26). Yet, current evidence suggests that although hypoglycemia exposure may impair executive function and visual–motor function in early childhood (27), it does not necessarily lead to lower educational achievement in mid-childhood (28). However, Sigal et al. reported that PSHI infants are at high risk of neurodevelopmental deficits and are more likely to perform below average (29).

We also show that the TID was substantially longer when clinicians intervened with DZX because treatment can only safely end when a formal fasting study is performed to document the resolution of the hyperinsulinemic state (15, 30). We appreciate that there may be concerns that our tapering method may lead to prolonged intervention. However, one published report recommends attempting withdrawal of DZX when the dosage is below 1 mg/kg/day. This study by Yorifuji et al. and ours considered safety a priority and an endpoint proven by a fasting study (15). Until an alternative method that satisfies these criteria is described, the tapering method may need to be necessarily adequate. The enthusiasm to initiate DZX is not often balanced with the necessary processes to stop it. Clinicians justify starting DZX by either citing the need to remove central lines to minimize the risk of sepsis or facilitating discharge to reduce the cost of inpatient care. However, our data suggest otherwise. Additionally, we appreciate that the responsiveness to DZX can reassure clinicians of the likely non-genetic nature of HH in SGA infants, as previously demonstrated by Arya et al. (31). Physicians often take calculated risks by stopping DZX without fasting studies to minimize the cost of care (30, 32). Whether this is appropriate is still being determined, as there needs to be a good clinical consensus in the literature on whether it is necessary to prove a resolution and how and when to stop DZX. Furthermore, there are differential costs of ready-made DZX preparations, which are highly expensive, versus compounded liquid formulations, which are less costly and less reliable but require pharmacy expertise (32, 33). We suggest that DZX therapy for SGA-HH should be regarded as a continuum of initiating DZX appropriately, monitoring glucose regularly, and stopping DZX safely (5). Clinical teams need to understand that costs extend beyond the initial LOS and that prolonging the duration of care may paradoxically add to psychological and economic burdens (34).

Strengths and limitations

To the best of our knowledge, this is the first study to compare clinically relevant outcomes of WW over pharmacological intervention in SGA-HH infants in a demographically and biochemically comparable cohort. We also determined the total duration of clinical commitment the patient and provider needed from diagnosis to HH resolution as determined by fasting studies. We acknowledge that two different pathways were employed during the study period, which limits consistency. However, because care pathways are so variable, presenting data from both pathways has provided practical, real-life information to clinical practitioners. To enhance the generalizability of our findings, we defined SGA by the Fenton 2013 criteria, where we identified 16% of live births as SGA even though we expected 10%. This is because birth norms from a Singapore cohort showed that the 10th percentile superimposed well with the Fenton chart between 25 and 39 weeks of gestation and then separated from 39 to 42 weeks, resulting in overestimation of SGA birth size in these latter babies (35). This limitation would apply to all populations, including Singapore. We did not perform genetic studies as all infants were either DZX responsive or underwent spontaneous resolution. We did not study the economic implications of these treatment modalities apart from establishing the duration of care. We acknowledge that the choice of treatment varied with time, with more recent cases being managed with WW. However, adjustment for time showed comparable findings. Due to the recent nature of this study, longitudinal follow-up is still in progress, and this information would be useful to determine the long-term outcomes.

Conclusion

This real-world observational study demonstrates that CLD and LOS are comparable among SGA-HH infants, whether DZX treatment is employed or not. Watchful waiting while supporting the metabolic needs of SGA infants with hyperinsulinism is a justifiable alternative to DZX therapy in infants with HH. Since fasting studies determine the resolution of HH, physicians should be aware that clinical intervention of DZX-treated SGA-HH infants extends beyond the initial LOS. However, as this was an observational study and SGA-HH is uncommon, our findings ought to be confirmed in a pragmatic multicenter randomized trial.

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 authors.

Ethics statement

The studies involving human participants were reviewed and approved by Singhealth Centralised Institutional Review Board, Singapore. Written informed consent from the participants' legal

guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

SC and FY conceived and designed the study, interpreted the data, and wrote the final manuscript; SJ-B prepared the preliminary draft of the manuscript, including references; VR designed the study and interpreted the data; SS conducted the statistical analysis and interpreted the data; MC designed the study and interpreted the data. SC and FY contributed equally. All authors critically revised the manuscript, agreed to be fully accountable for ensuring the integrity and accuracy of the work. 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.2023.1163591/full#supplementary-material

SUPPLEMENTARY TABLE 1
Clinical outcomes of infants comparing GCP vs FCP.

References

1. Hussain K, Aynsley-Green A. The effect of prematurity and intrauterine growth restriction on glucose metabolism in the newborn. *Neoreviews* (2004) 5:365–9. doi: 10.1542/neo.5-9-e365

2. Srinivasan G, Pildes RS, Cattamanchi G, Voora S, Lilien LD. Plasma glucose values in normal neonates: a new look. *J Pediatr* (1986) 109:114–7. doi: 10.1016/s0022-3476(86)80588-1

3. Collins JE, Leonard JV, Teale D, Marks V, Williams DM, Kennedy CR, et al. Hyperinsulinaemic hypoglycaemia in small for date babies. *Arch Dis Child* (1990) 65:1118–20. doi: 10.1136/adc.65.10.1118

4. Collins JE, Leonard JV. Hyperinsulinism in asphyxiated and small for date infants with hypoglycemia. *Lancet* (1984) 2:311–3. doi: 10.1016/s0140-6736(84)92685-0

5. Chandran S, PR R, Mei Chien C, Saffari SE, Rajadurai VS, Yap F. Safety and efficacy of low-dose diazoxide in small-for-gestational-age infants with hyperinsulinaemic hypoglycaemia. *Arch Dis Child Fetal Neonatal Ed* (2022) 107:359–63. doi: 10.1136/archdischild-2021-322845

6. Thornton P, De Leon DD. What's in a name? that which we call perinatal stress hyperinsulinism by any other name would smell so bitter. *J Perinatol* (2021) 41:2110. doi: 10.1038/s41372-021-01056-z

7. Hoe FM, Thornton PS, Wanner LA, Steinkrauss L, Simmons RA, Stanley CA. Clinical features and insulin regulation in infants with a syndrome of prolonged neonatal hyperinsulinism. *J Pediatr* (2006) 148:207–12. doi: 10.1016/j.jpeds.2005.10.002

8. Fafoula O, Alkhayyat H, Hussain K. Prolonged hyperinsulinaemic hypoglycaemia in newborns with intrauterine growth retardation. *Arch Dis Child Fetal Neonatal Ed* (2006) 91:F467. doi: 10.1136/adc.2006.095919

9. Hawdon JM, Beer J, Sharp D, Upton M. NHS Improvement patient safety programme 'Reducing term admissions to neonatal units'. neonatal hypoglycaemia: learning from claims. *Arch Dis Child Fetal Neonatal Ed* (2017) 102:F110–15. doi: 10.1136/archdischild-2016-310936

10. Prado LA, Castro M, Weisz DE, Jain A, Belik J. Necrotising enterocolitis in newborns receiving diazoxide. *Arch Dis Child Fetal Neonatal Ed* (2021) 106:306–10. doi: 10.1136/archdischild-2020-319057

11. Herrera A, Vajravelu ME, Givler S, Mitteer L, Avitabile CM, Lord K, et al. Prevalence of adverse events in children with congenital hyperinsulinism treated with diazoxide. *J Clin Endocrinol Metab* (2018) 103:4365–72. doi: 10.1210/jc.2018-01613

12. Fenton TR, Kim JH. A systematic review and meta-analysis to revise the fenton growth chart for preterm infants. *BMC Pediatr* (2013) 13:59. doi: 10.1186/1471-2431-13-59

13. Kapoor RR, Flanagan SC, James C, Shield J, Ellard S, Hussain K. Hyperinsulinemic hypoglycemia. *Arch Dis Child* (2009) 94(6):450–7. doi: 10.1007/s13312-015-0772-1

14. Senniappan S, Arya VB, Hussain K. The molecular mechanisms, diagnosis and management of congenital hyperinsulinism. *Indian J Endocr Metab* (2013) 17:19–30. doi: 10.4103/2230-8210.107822

15. Yorifuji T, Horikawa R, Hasegawa T, Adachi M, Soneda S, Minagawa M, et al. Clinical practice guidelines for congenital hyperinsulinism. *Clin Pediatr Endocrinol* (2017) 26:127–52. doi: 10.1297/cpe.26.127

16. Chandran S, Siew JX, Rajadurai VS, Lim RWS, Chu MC, Yap F. A feed-centric hypoglycaemia pathway ensures appropriate care escalation in at-risk infants. *BMJ Open Qual* (2021) 10:e001296. doi: 10.1136/bmjoq-2020-001296

17. Balachandran B, Mukhopadhyay K, Sachdeva N, Walia R, Attri SV. Randomised controlled trial of diazoxide for small for gestational age neonates with hyperinsulinaemic hypoglycaemia provided early hypoglycaemic control without adverse effects. *Acta Paediatr* (2018) 107:990–5. doi: 10.1111/apa.14252

18. Keyes ML, Healy H, Sparger KA, Orth LE, Geha M, Roumiantsev S, et al. Necrotizing enterocolitis in neonates with hyperinsulinemic hypoglycemia treated with diazoxide. *Pediatrics* (2021) 147:e20193202. doi: 10.1542/peds.2019-3202

19. Hastings LA, Preddy J, McCready M, Neville K, Verge CF. Pericardial effusion associated with diazoxide treatment for congenital hyperinsulinism. *Horm Res Paediatr* (2020) 93:206–11. doi: 10.1159/000507624

20. Timlin MR, Black AB, Delaney HM, Matos RI, Percival CS. Development of pulmonary hypertension during treatment with diazoxide: a case series and literature review. *Pediatr Cardiol* (2017) 38:1247–50. doi: 10.1007/s00246-017-1652-3

21. Chen SC, Dastamani A, Pintus D, Yau D, Aftab S, Bath L, et al. Diazoxide-induced pulmonary hypertension in hyperinsulinaemic hypoglycaemia: recommendations from a multicentre study in the United Kingdom. *Clin Endocrinol (Oxf)* (2019) 91:770–5. doi: 10.1111/cen.14096

22. Gray D, Dudash K, Escobar C, Freel C, Harrison T, McMillan C, et al. Prevalence and safety of diazoxide in the neonatal intensive care unit. *J Perinatol* (2018) 38:1496–502. doi: 10.1038/s41372-018-0218-4

23. Feng ZP, Sun HS. ATP-sensitive potassium channels (KATP) play a role in hypoxic preconditioning against neonatal hypoxic–ischemic brain injury. In: Lapchak P, Zhang J, editors. *Neuroprotective therapy for stroke and ischemic disease. springer series in translational stroke research*. Cham: Springer (2017). p. 185–201.

24. Vollmer B, Edmonds CJ. School age neurological and cognitive outcomes of fetal growth retardation or small for gestational age birth weight. *Front Endocrinol (Lausanne)* (2019) 10:186. doi: 10.3389/fendo.2019.00186

25. Sacchi C, Marino C, Nosarti C, Vieno A, Visentin S, Simonelli A. Association of intrauterine growth restriction and small for gestational age status with childhood cognitive outcomes: a systematic review and meta-analysis. *JAMA Pediatr* (2020) 174:772–81. doi: 10.1001/jamapediatrics.2020.1097

26. Burns CM, Rutherford MA, Boardman JP, Cowan FM. Patterns of cerebral injury and neurodevelopmental outcomes after symptomatic neonatal hypoglycemia. *Pediatrics* (2008) 122:65–74. doi: 10.1542/peds.2007-2822

27. McKinlay CJD, Alsweiler JM, Anstice NS, Burakevych N, Chakraborty A, Chase JG, et al. Association of neonatal glycemia with neurodevelopmental outcomes at 4.5 years. *JAMA Pediatr* (2017) 171:972–83. doi: 10.1001/jamapediatrics.2017.1579

28. Shah R, Dai DWT, Alsweiler JM, Brown GTL, Chase JG, Gamble GD, et al. Association of neonatal hypoglycemia with academic performance in mid-childhood. *JAMA* (2022) 327:1158–70. doi: 10.1001/jama.2022.0992

29. Sigal WM, Alzahrani O, Guadalupe GM, Guzman H, Radcliffe J, Thomas NH, et al. Natural history and neurodevelopmental outcomes in perinatal stress induced hyperinsulinism. *Front Pediatr* (2022) 10:999274. doi: 10.3389/fped.2022.999274

30. 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:dgaa543. doi: 10.1210/clinem/dgaa543

31. Arya VB, Flanagan SE, Kumaran A, Shield JP, Ellard S, Hussain K, et al. Clinical and molecular characterisation of hyperinsulinaemic hypoglycaemia in infants born small-for-gestational age. *Arch Dis Child Fetal Neonatal Ed* (2013) 98:F356–8. doi: 10.1136/archdischild-2012-302880

32. Raisingani M, Brar PC. Characterization of the duration of treatment with diazoxide in infants with prolonged hyperinsulinism (PHI). *J Pediatr Endocrinol Metab* (2019) 32:1241–5. doi: 10.1515/jpem-2019-0066

33. Friciu M, Zaraa S, Roullin VG, Leclair G. Stability of diazoxide in extemporaneously compounded oral suspensions. *PloS One* (2016) 11:e0164577. doi: 10.1371/journal.pone.0164577

34. 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) 17:61. doi: 10.1186/s13023-022-02214-y

35. Poon WB, Fook-Chong SM, Ler GY, Loh ZW, Yeo CL. Creation and validation of the Singapore birth nomograms for birth weight, length and head circumference based on a 12-year birth cohort. *Ann Acad Med Singap* (2014) 43:296–304. doi: 10.47102/annals-acadmedsg.V43N6p296

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Abnormal glucose homeostasis and fasting intolerance in patients with congenital porto-systemic shunts

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In physiological glucose homeostasis, the liver plays a crucial role in the extraction of glucose from the portal circulation and storage as glycogen to enable release through glycogenolysis upon fasting. In addition, insulin secreted by the pancreas is partly eliminated from the systemic circulation by hepatic first-pass. Therefore, patients with a congenital porto-systemic shunt present a unique combination of (a) postabsorptive hyperinsulinemic hypoglycaemia (HH) because of decreased insulin elimination and (b) fasting (ketotic) hypoglycaemia because of decreased glycogenolysis. Patients with porto-systemic shunts therefore provide important insight into the role of the portal circulation and hepatic function in different phases of glucose homeostasis.

KEYWORDS

hypoglycaemia, hyperinsulinism, congenital porto-systemic shunt, portal circulation, glucose metabolism, insulin, congenital hyperinsulinism

Introduction

The liver has multiple roles in endocrine physiology, which are increasingly recognized (1). These roles include hormone synthesis (e.g. IGF-1), degradation (e.g. insulin or sex steroids), precursor synthesis (e.g. cholesterol and lipoproteins for glucocorticoid synthesis), and binding protein synthesis. In normal physiology, the liver receives 25% of its afferent blood supply from the hepatic artery, and 75% from the portal vein (2). Necessary endocrine metabolites enter the liver either by the portal vein or by the hepatic

artery. In patients with a congenital portosystemic shunt (CPSS), the liver is bypassed to varying degrees, leading to metabolite and hormonal abnormalities, including hypoglycaemia, hyperammonemia, increased blood bile acids, hypothyroidism and hyperandrogenism, among others (3).

Although hypoglycaemia is a relatively common metabolic abnormality in pediatrics, awareness of CPSS as an underlying cause is low, as illustrated by the fact that CPSS was not a differential diagnosis in a recent review of hypoglycaemia (4). Yet, patients with CPSS are at risk for a unique combination of postabsorptive HH and fasting (ketotic) hypoglycaemia.

Therefore, the primary aim of this manuscript is to summarize the current knowledge of hypoglycaemia in CPSS patients, and the secondary aim of this review is to go deeper into glucose metabolism in CPSS, which in turn enhances our understanding of the liver's role in glucose and insulin physiology.

Case report

An 8-year-old child had experienced loss of consciousness after a 15-hour fast during an infectious episode at 9 months of age. Despite normal development and physical examination with no organomegaly, a fasting test revealed hypoglycaemia of 2.6 mmol/L (47 mg/dl) and ketones of 2.2 mmol/L. Glucose levels rose to 11.8 mmol/L (212 mg/dl) upon feeding, leading to suspicion of glycogen storage disease type 0, which was ruled out through genetic testing. An oral glucose tolerance test was performed to investigate the response to feeding and test gluconeogenic and mitochondrial function, revealing hypoglycaemia of 1.9 mmol/L (34 mg/dl) after 120 minutes with a peak glucose of 10.7 mmol/L (193 mg/dl). Continuous glucose sensing showed regular nocturnal hypoglycaemia, and treatment with twice daily corn starch in a gradually increased amount improved wellbeing. Metabolic analyses showed mildly increased ammonia (<100 umol/l) and purines, but genetic investigation was non-contributory. Untargeted metabolomics in a research setting revealed increased bile salt concentrations together with hypoglycaemia and hyperammonemia, leading to a diagnosis of CPSS, as was confirmed by ultrasound imaging. Our experience with similar cases has led to considering CPSS in the differential diagnosis of unexplained hypoglycaemia.

Patients with CPSS and hypoglycaemia

The first case of HH in CPSS was reported in 1984 by Gouin and Duprey (5, 6). They described a 63-year-old woman with recurrent malaise due to hypoglycaemic episodes, which was ultimately attributed to a portosystemic shunt thought to be congenital in origin. The patient's glycoregulation showed impaired glucose tolerance, followed by post-stimulatory hypoglycaemia with persistent hyperinsulinemia. Since then, more than 10 cases have been reported by various authors (3, 7–13). In many of these cases, hypoglycaemia was the presenting symptom. However, it is currently unknown how many patients with CPSS suffer from HH, as screening for hypoglycaemia is not standard in CPSS patients. For instance, Sokollik et al. (14) did not report hypoglycaemic episodes in their case series of 22 patients, while Baiges et al. (15) did not find any abnormalities in glucose homeostasis in a series of 66 adults with extra-hepatic CPSS. Similarly, a small series of infants with intra-hepatic CPSS did not mention HH. However, Xu et al. presented a neonatal cohort of 16 patients with CPSS, 40% of whom presented with hypoglycaemia as a clinical symptom (9).

Glucose homeostasis

Ruderman (16) divided glucose homeostasis into five phases starting from the moment of carbohydrate ingestion: absorptive, post-absorptive, and early, intermediate, and prolonged starvation. The first two phases are essential to understanding glucose metabolism in the setting of CPSS.

In the absorptive phase, glucose and insulin are the most crucial components. Digestive tract glucosidases process ingested carbohydrates. The resulting monosaccharides are transported to the liver via the portal circulation, where they enter hepatocytes through insulin-mediated transport. Glycogen synthase initially converts them into glycogen, and excessive carbohydrate consumption leads to lipogenesis. Under normal conditions, one-third of the glucose in the portal vein is taken up by the liver, and the rest of it is delivered to the rest of the body through arterial blood (17).

Insulin levels rise in response to elevated blood glucose and regulate hepatic glucose uptake. In parallel, pancreatic beta-cells act as glucose sensors, linking changes in arterial blood glucose concentration to the rate of insulin secretion. Pre-proinsulin processes to its mature form, generating c-peptide. Typically, insulin production occurs in less than two hours. Roughly 75 to 95% of total insulin is stored within beta-cells at some distance from the cell membrane, while a smaller part of the insulin is packed in granules that are ready to go. This efficient organization likely underlies the biphasic nature of glucose-evoked insulin secretion that is seen *in vitro*, with a rapid first phase that lasts up to 10 min followed by second phase (18).

The human pancreas secretes approximately 30 units of insulin per day into the portal circulation. Insulin that enters the hepatic sinusoid through the portal circulation accesses hepatocytes through the fenestrated sinusoidal endothelium. It binds to the insulin receptor and, after internalization, is degraded by insulin-degrading enzymes. Normal hepatic insulin clearance is around 50-80% of daily production (19). Peripheral insulin concentration increases within 8-10 minutes after food ingestion, peaks at 30-45 minutes, and rapidly declines to baseline values by 90-120 minutes postprandially (19, 20). Insulin's primary action is to stimulate glucose uptake and promote glycogen synthesis in the liver and muscles.

When it was demonstrated that oral glucose intake results in a greater and longer-lasting rise in plasma insulin compared to an

intravenous administration of the same dose, scientists hypothesized that there must be a gastro-intestinal factor triggered by alimentary glucose. This was later termed the incretin effect (21), with the two most important incretins being Glucagon-like peptide 1 (GLP-1) and GIP, both of which independently contribute to insulin release.

GLP-1 is produced as proglucagon and is primarily generated in the entero-endocrine L cells that are found in the intestine, especially in the distal ileum. The production of GLP-1 is stimulated by the consumption of various nutrients, particularly glucose and fat, as well as bile acids. The GLP-1 receptor is found in numerous organs and stimulates insulin secretion in a glucose-dependent manner. However, GLP-1 is rapidly degraded *in vivo* by dipeptidyl peptidase-4 (DPP-4), an enzyme that is highly expressed in the liver (22). DPP-4 is a crucial determinant in regulating the biological activity of incretins. Thus, liver disease or bypass may impact fasting glucose regulation both through diminished GLP-1 degradation and altered bile acid signaling given the partial interruption in the enterohepatic circulation that occurs in CPSS (23).

In the normal post-absorptive phase, insulin action is suppressed when circulating glucose levels decrease, and the body relies on endogenous glucose production (EGP). EGP first utilizes glycogenolysis (second phase), followed by gluconeogenesis in early starvation (third phase), fatty acid oxidation in intermediate starvation (fourth phase), and ketogenesis and ketolysis after prolonged starvation (fifth phase). A large portion of this endogenously produced glucose is used to provide energy for the brain. The ratio of brain weight to body weight is highest in young children (24), and studies have shown that 3-4 year old children have a glucose appearance rate that is approximately 3 times higher (6-7 mg/kg/min) than in adults (25). As a result, liver glycogen stores are depleted more quickly in children, which can result in the formation of ketone bodies after a few hours of fasting (26), indicating intermediate starvation after a brief period.

Glucagon plays a critical role in maintaining glucose homeostasis in the post-absorptive phase (27). Its primary secretory stimulus is a low plasma glucose concentration, although other variables such as the stress related to trauma or hypoxia may act as triggers (28). Pancreatic glucagon secretion leads to a kinase-mediated release of glucose from stored glycogen. Proglucagon processing leads to the production of GLP-1 and -2 in intestinal L-cells, as well as the production of glucagon in pancreatic alpha cells (29). Glucagon is transported through the portal circulation to the liver, where it exerts its effects via a specific extracellular receptor.

Glucose metabolism in CPSS

In CPSS patients, hepatic bypass can cause a rapid spike in plasma glucose levels due to the postprandial entry of glucose into the systemic circulation. This effect has been observed in OGTTs and fasting tests, where patient glucose levels spiked significantly (3). This spike in glucose levels is followed by hypoglycaemia, occurring 90-120 minutes after carbohydrate-rich meals that are high in fast-acting carbohydrates and low in other macro-nutrients. The increased insulin levels due to hepatic bypass are incompletely degraded, leading to this hypoglycaemia. It is possible that a decrease in GLP-1 breakdown might help mitigate this effect. This HH might be considered one of the metabolic hallmarks of CPSS.

Additionally, smaller children may be more vulnerable to fasting-induced hypoglycaemia with ketone production, as hepatic glycogenesis may be less efficient in this population. Possibly, altered glucagon metabolism also contributes to a different fasting ability.

It is important to note that the clinical picture of CPSS can be diverse and may vary depending on the quantitative portal flow and the presence of additional metabolites and pathways affected by portocaval shunting, such as fatty acid oxidation and amino acid metabolism, which are beyond the scope of this review. CPSS patients can present a wide spectrum of relatively nonspecific symptoms, signs and complications. These may occur throughout life and include unexplained neurocognitive dysfunction and behavioral problems, neonatal cholestasis, and galactosemia. Some of these symptoms and signs are mimicking several rare inherited metabolic disorders, in which a substrate shunt is caused by a transporter or enzyme deficiency at a cellular level. Table 1 summarizes the key-features of four inherited metabolic disorders in very different metabolic pathways, that display interesting similarities and differences with CPSS. Both in these metabolic patients and CPSS patients, the metabolic profile depends highly on the timing of blood sampling with respect to the last meal.

Diagnosing hypoglycaemia in CPSS

Acute hypoglycaemia is a common pediatric metabolic emergency with a wide range of possible causes. Rare conditions like CPSS and inherited metabolic disorders are not always taken into consideration in the differential diagnosis. Furthermore, the definition of childhood hypoglycaemia is not always clear cut. The normal range for fasting glucose levels is between 3.5 mmol/L and 5.5 mmol/L (63 mg/dL and 100 mg/dL) (30). Generally, for neonates over 48 hours old, infants, and young children, a glucose level below 3.3 mmol/L (60 mg/dL) is considered too low (31). However, it is worth noting that healthy children between the ages of 8 and 15 can occasionally have glucose levels ≤ 3.3 mmol/L or 60 mg/dL in 0.2% of the time on continuous glucose monitoring (CGM), and ≤ 3.9 mmol/L or 70 mg/dL in nearly 2% of the time (32–34). The presence of Whipple's triad (classical symptoms of hypoglycaemia, symptoms at the time of a low blood glucose concentration, and relief of symptoms after the increase of the glucose level), when present, can be helpful in diagnosing hypoglycaemia in older children (30).

It is essential to monitor patients at risk for hypoglycaemia, especially in pre-school children, as they are vulnerable to the consequences of hypoglycaemia on their developing central nervous system. Patients with congenital hyperinsulinism are known to be extremely susceptible to the effects of hypoglycaemia on the brain because insulin also decreases the production of

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TABLE 1 Examples of metabolic diseases with molecular glucose shunting.

Name	Glucose transporter 2 deficiency	Hepatic glycogen synthase deficiency	Glutamate dehydrogenase superactivity	Transmembrane protein 70 deficiency
Disease abbreviation	FBS (Fanconi-Bickel Syndrome)	GSD-0a	GLUD1	NME (Neonatal Mitochondrial Encephalopathy)
Gene	*SLC2A2*	*GYS2*	*GLUD1*	*TMEM70*
OMIM	# 227810	# 240600	# 606762	# 614052
Inheritance	Autosomal recessive	Autosomal recessive	Autosomal dominant	Autosomal recessive
Hepatocyte location	Cell membrane	Cytosol	Mitochondrial	Mitochondrial
Clinical symptoms	Hepatomegaly n -↑↑↑ Renal tubulopathy, generalized ± Short stature n-+++ Nefromegaly Reduced bone density Rickets Thin limbs	Hepatomegaly n Hypoglycemia, fasting ± Seizures ±	Epilepsy, generalized ± - ++ Hyperinsulinism ± - ++ Hypoglycemia + - ++ Hypoglycemia, hypoketotic + - ++ Leucine sensitivity causing hypoglycemia ++	Cardiomyopathy, hypertrophic + Encephalopathy + Failure to thrive + Hypotonia, muscular-axial + Ketonuria, pronounced during crisis + Lactic acidosis ± - + Metabolic acidosis ± - + Psychomotor delay ± - + Low birth weight Facial dysmorphic features Ataxia in those who survive Intention tremor in those who survive Leukoencephalopathy
Biochemical markers	Plasma glucose: - Fed n-↑ - Fasted n-↓ Plasma galactose ± Plasma uric acid ↑ Serum: - AST/ALT n -↑ - Cholesterol n -↑ - Triglycerides n -↑ - AF n -↑ Urine: - Glucose ↑-↑↑↑ - Calcium n -↑ - Phosphate n -↑ - Amino acids n -↑	Plasma glucose: - Fed n -↑ - Fasted n -↓ Plasma lactate: - Fed n -↑ Plasma ketones: - Fasted n -↑ Urine ketones: - Fasted n -↑	Plasma glucose ↓ Serum free fatty acids ↓ Blood/plasma ammonia - Fasted ↑ Plasma/urine ketones ↓	Plasma amino acids: Ala ↑-↑↑, Cit n -↑, Glu n-↑ Plasma CK n -↑↑ Plasma lactate Glucose ↑-↑↑↑ Blood ammonia n -↑↑ Anion gap ↑ Urine: - 3-methylglutaconic acid ↑-↑↑ - Orotic acid n-↑ - Uric acid n-↑↑

(n, normal; ↑, mildly increased; ↑↑, increased; ↑↑↑, severely increased; ↓, decreased; ±, sometimes present; +, often present; ++, always.

ketones, the alternative fuel for the brain (35, 36). This is further illustrated by the high prevalence of neurocognitive dysfunction in these patients (37). In patients with CPSS, the symptoms of hypoglycaemia can be difficult to distinguish from symptoms related to minimal hepatic encephalopathy in the presence of elevated plasma ammonia, one of the other metabolic characteristics of patients with CPSS.

Patients at risk for hypoglycaemia can be monitored using frequent finger prick blood glucose testing, oral glucose tolerance test, fasting test, or continuous glucose monitoring (CGM). CGM use is rapidly expanding due to its continuous measurements and relatively uninvasive nature. In patients with diabetes mellitus, CGM is the standard of care. For the early recognition and reduction of HH, CGM is also becoming increasingly established (38). In rare or unexplained diseases with hypoglycaemia, CGM can provide additional information, helping to determine the etiology and to optimize treatment for the individual patient, as recently reported for patients with hepatic glycogen storage disease (39).

The oral glucose tolerance test with measurement of glucose and insulin at every time point is most frequently used to establish the diagnosis of hypoglycemia in CPSS (3, 7, 8, 10, 12), with a typical pattern of postprandial hyperglycemia followed by hypoglycemia (Figure 1). However, glucose and insulin are only measured every 30 minutes during 2.5-3 hours after glucose ingestion. CGM has the advantage of measuring glucose levels for several days in daily life practice, providing a better understanding of the fluctuations of glucose levels. This ultimately leads to a better understanding of the disease and the optimization of management for each individual patient.

Treatment of hypoglycaemia in CPSS

Symptomatic CPSS need to be closed either using an endovascular or surgical approach. This has been documented to resolve hypoglycaemia (10–13). Dietary management of

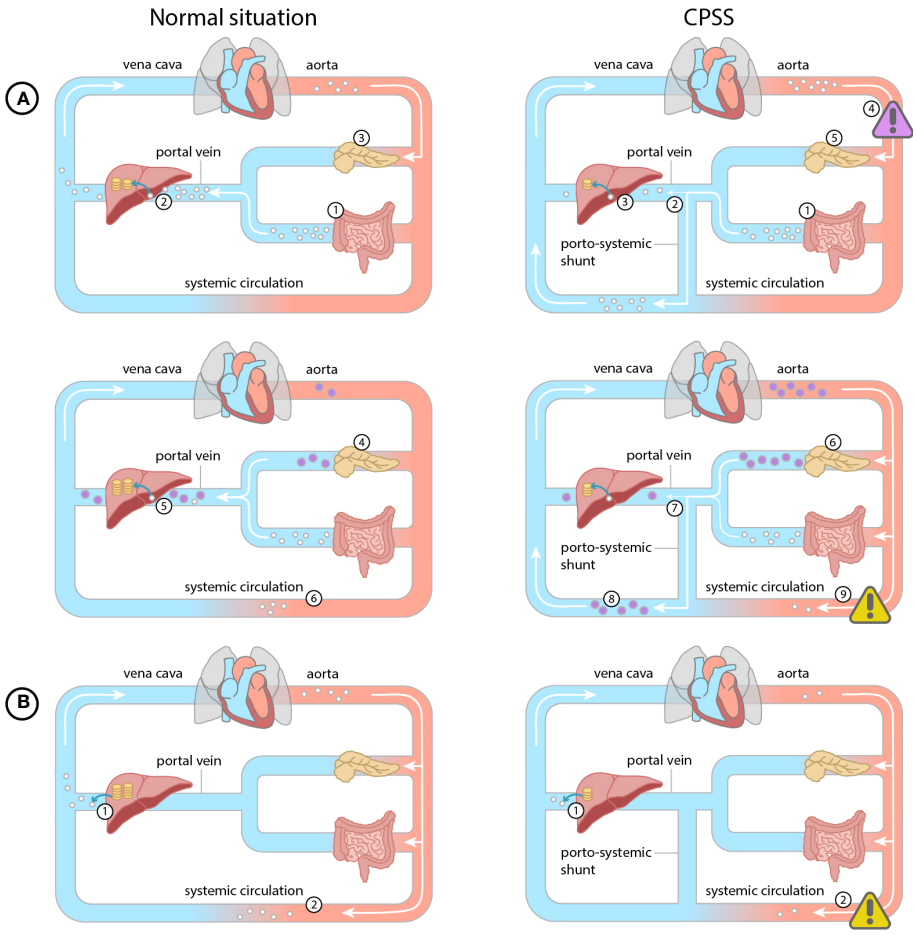

FIGURE 1
Normal situation (glucose = white dot, insulin = purple dot, glycogen = yellow coin): **(A)** Post prandial. 1) Glucose is absorbed from the intestines into the mesenteric veins. 2) Glucose is converted and stored as glycogen in the liver during the first pass effect. 3) Pancreatic beta cells detect the increased glucose levels. 4) Pancreatic beta cells secrete insulin according to glucose levels. 5) Hepatic insulin metabolism reduces systemic insulin levels. **(B)** During fasting. 1) Stored glycogen is converted to glucose and enters the bloodstream. 2) This mechanism prevents hypoglycemia during fasting. CPSS **(A)** Post prandial glucose. 1) Glucose is absorbed from the intestines into the mesenteric veins. 2) Due to the porto-systemic shunt the glucose partly bypasses the liver, 3) causing less glucose to be stored as glycogen in the liver 4) and a systemic hyperglycemia (purple warning sign). 5) Pancreatic beta cells detect the increased glucose levels. 6) Pancreatic beta cells excrete more insulin. 7) The insulin bypasses the hepatic metabolism directly flowing into the systemic circulation 8) leading to systemic hyperinsulinemia. 9) Hyperinsulinemia results in a late hypoglycemia in end-organs (yellow warning sign). **(B)** During fasting. 1) Due to reduced glycogen storage in the liver, significantly less glycogen can be converted to glucose to enter the bloodstream 2) resulting in a hypoglycemia in end-organs (yellow warning sign).

hypoglycaemia can be considered in rare cases of contraindication to closure or as a bridge to closure. Two studies have reported on the management of HH related to CPSS (8, 11). The dietary approach involves a diet low in simple carbohydrates and rich in complex carbohydrates, with the prevention of longer fasting. Specifically, the use of galactose-free milk and corn starch is recommended (8). Drug treatment may consist of alpha-glucosidase inhibitors or diazoxide, although only one patient received diazoxide in the study of Weigert et al. (11).

Meals with slow-acting carbohydrates with a low glycemic index may prevent insulin spiking in these patients. Adding ultra-slow acting carbohydrates, such as corn starch or glycosade, to the diet can help prevent extremely low glucose levels and hypoglycaemia after a night of fasting. Corn starch therapy should be initiated with small doses, gradually increased, and carefully monitored, as the enzyme needed for intestinal digestion of cornstarch may not be fully present before 2 years of age. The effects on glucose homeostasis, fasting tolerance, and symptoms (including mood and growth) should be carefully balanced against side effects, such as intestinal gas, bloating, and diarrhea.

Diazoxide is one of the most potent agonists of the K+ ATP channels on the insulin-producing beta-cells of the pancreas. Its activation reduces insulin secretion and theoretically, it could be used to prevent hyperinsulinism and hypoglycaemia in CPSS patients. However, it may further increase the high spikes in glucose, potentially aggravating the fluctuations. There have been some reports with variable effects on postprandial hypoglycaemia. The main side effect is fluid retention, sometimes complicated by hypertrichosis or pulmonary hypertension (PoPH). Given the association between PoPH and CPSS, avoiding diazoxide seems reasonable. Alpha-glucosidase inhibitors are another treatment option, although their effectiveness in CPSS is not well established.

Conclusions and perspectives

Congenital porto-systemic shunts are vascular anomalies that can affect glucose and insulin metabolism, and are most typically characterized by episodes of HH and variable fasting intolerance. Despite their rarity, they should be considered as a potential cause of hypoglycaemia and are easily diagnosed using Doppler ultrasound. In patients diagnosed with CPSS we advise to screen patients for hypoglycaemia with continuous glucose monitoring or oral glucose tolerance testing with glucose and insulin at every time point. Repeated hypoglycaemia during childhood can have serious neurological and cognitive consequences, particularly if there is not enough ketone production to compensate. Therefore, it is recommended to evaluate glucose and ketone levels after fasting and carbohydrate intake in these patients. Shunt closure is the ultimate solution, but dietary management can also play a significant role in stabilizing glucose levels as a bridge to closure or in rare cases where shunt closure is contraindicated. The intricacies underlying varying individual presentations and tolerance to HH remain to be elucidated, as do the impact of portosystemic bypass on other metabolic pathways involved in buffering the stress of fasting.

Author contributions

MA, VM and HD drafted and corrected the manuscript PS corrected the manuscript and provided clinical input TD contributed the table and made additions to the manuscript from the metabolic perspective SF provided clinical details from the case and corrected the manuscript JJ performed and interpreted the metabolomic data and provided specific knowledge in the metabolomics field. All authors contributed to the article and approved the submitted version.

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Conflict of interest

VM consults for Mirum Pharmaceuticals, Albireo, and AstraZeneca.

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References

1. Rhyu J, Yu R. Newly discovered endocrine functions of the liver. *World J Hepatol* (2021) 13:1611–28. doi: 10.4254/wjh.v13.i11.1611

2. Eipel C, Abshagen K, Vollmar B. Regulation of hepatic blood flow: the hepatic arterial buffer response revisited. *World J Gastroenterol* (2010) 16:6046–57. doi: 10.3748/wjg.v16.i48.6046

3. Bas S, Guran T, Atay Z, Haliloglu B, Abalı S, Turan S, et al. Premature pubarche, hyperinsulinemia and hypothyroxinemia: novel manifestations of congenital portosystemic shunts (Abernethy malformation) in children. *Horm Res Paediatr* (2015) 83:282–7. doi: 10.1159/000369395

4. 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

5. Gouin B, Le Gal J, Duprey J, Sanson J. Congenital intrahepatic portacaval anastomosis: analysis of manifested glucose abnorMalities. *Gastroenterol Clin Biol* (1984) 8:464–8.

6. Duprey J, Gouin B, Benazet MF, Le Gal J. Glucose intolerance and post-stimulatory hypoglycemia secondary to a probably congenital intrahepatic portacaval anastomosis. *Ann Med Interne (Paris)* (1985) 136:655–8.

7. Satoh M, Yokoya S, Hachiya Y, Hachiya M, Fujisawa T, Hoshino K, et al. Two hyperandrogenic adolescent girls with congenital portosystemic shunt. *Eur J Pediatr* (2001) 160:307–11. doi: 10.1007/s004310000539

8. Yoshii K, Noda M, Naiki Y, Horikawa R. Portosystemic shunt as a cause of congenital hyperinsulinemic hypoglycemia. *Pediatr Int* (2017) 59:512–4. doi: 10.1111/ped.13245

9. Xu S, Zhang P, Hu L, Zhou W, Cheng G. Case report: clinical features of congenital portosystemic shunts in the neonatal period. *Front Pediatr* (2021) 9:778791. doi: 10.3389/fped.2021.778791

10. Senniappan S, Pitt K, Shah P, Arya V, Jaiswal S, Haddad M, et al. Postprandial hyperinsulinaemic hypoglycaemia secondary to a congenital portosystemic shunt. *Horm Res Paediatr* (2015) 83:217–20. doi: 10.1159/000369014

11. Weigert A, Bierwolf J, Reutter H, Gembruch U, Woelfle J, Ganschow R, et al. Congenital intrahepatic portocaval shunts and hypoglycemia due to secondary hyperinsulinism: a case report and review of the literature. *J Med Case Rep* (2018) 12:336. doi: 10.1186/s13256-018-1881-y

12. Timpanaro T, Passanisi S, Sauna A, Trombatore C, Pennisi M, Petrillo G, et al. Congenital portosystemic shunt: our experience. *Case Rep Pediatr* (2015) 2015:691618. doi: 10.1155/2015/691618

13. Ghasemi-Rad M, Smuclovisky E, Cleveland H, Hernandez JA. Endovascular treatment of a portosystemic shunt presenting with hypoglycemia; case presentation and review of literature. *Clin Imaging* (2022) 83:131–7. doi: 10.1016/j.clinimag.2021.12.020

14. Sokollik C, Bandsma RHJ, Gana JC, van den Heuvel M, Ling SC. Congenital portosystemic shunt: characterization of a multisystem disease. *J Pediatr Gastroenterol Nutr* (2013) 56:675–81. doi: 10.1097/MPG.0b013e31828b3750

15. Baiges A, Turon F, Simón-Talero M, Tasayco S, Bueno J, Zekrini K, et al. Congenital extrahepatic portosystemic shunts (Abernethy malformation): an international observational study. *Hepatology* (2020) 71:658–69. doi: 10.1002/hep.30817

16. Ruderman NB. Muscle amino acid metabolism and gluconeogenesis. *Annu Rev Med* (1975) 26:245–58. doi: 10.1146/annurev.me.26.020175.001333

17. Tokarz VL, MacDonald PE, Klip A. The cell biology of systemic insulin function. *J Cell Biol* (2018) 217:2273–89. doi: 10.1083/jcb.201802095

18. Wang Z, Thurmond DC. Mechanisms of biphasic insulin-granule exocytosis - roles of the cytoskeleton, small GTPases and SNARE proteins. *J Cell Sci* (2009) 122:893–903. doi: 10.1242/jcs.034355

19. Bergman RN, Kabir M, Ader M. The Physiology of Insulin Clearance. *Int J Mol Sci* (2022) 23(3):1826. doi: 10.3390/ijms23031826

20. Najjar SM, Perdomo G. Hepatic insulin clearance: mechanism and physiology. *Physiol (Bethesda)* (2019) 34:198–215. doi: 10.1152/physiol.00048.2018

21. Creutzfeldt W. The [pre-] history of the incretin concept. *Regul Pept* (2005) 128:87–91. doi: 10.1016/j.regpep.2004.08.004

22. Mentlein R, Gallwitz B, Schmidt WE. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7-36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur J Biochem* (1993) 214:829–35. doi: 10.1111/j.1432-1033.1993.tb17986.x

23. Itou M, Kawaguchi T, Taniguchi E, Sata M. Dipeptidyl peptidase-4: a key player in chronic liver disease. *World J Gastroenterol* (2013) 19:2298–306. doi: 10.3748/wjg.v19.i15.2298

24. 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) 26:1016–23. doi: 10.2337/diab.26.11.1016

25. Huidekoper HH, Ackermans MT, Ruiter AFC, Sauerwein HP, Wijburg FA. Endogenous glucose production from infancy to adulthood: a non-linear regression model. *Arch Dis Child* (2014) 99:1098–102. doi: 10.1136/archdischild-2013-305718

26. Neville KA, Sandeman DJ, Rubinstein A, Henry GM, McGlynn M, Walker JL. Prevention of hyponatremia during maintenance intravenous fluid administration: a prospective randomized study of fluid type versus fluid rate. *J Pediatr* (2010) 156:313–9.e1–2. doi: 10.1016/j.jpeds.2009.07.059

27. Habegger KM, Heppner KM, Geary N, Bartness TJ, DiMarchi R, Tschöp MH. The metabolic actions of glucagon revisited. *Nat Rev Endocrinol* (2010) 6:689–97. doi: 10.1038/nrendo.2010.187

28. Bloom SR, Edwards AV, Hardy RN. The role of the autonomic nervous system in the control of glucagon, insulin and pancreatic polypeptide release from the pancreas. *J Physiol* (1978) 280:9–23. doi: 10.1113/jphysiol.1978.sp012369

29. Rouillé Y, Westermark G, Martin SK, Steiner DF. Proglucagon is processed to glucagon by prohormone convertase PC2 in alpha TC1-6 cells. *Proc Natl Acad Sci U.S.A.* (1994) 91:3242–6. doi: 10.1073/pnas.91.8.3242

30. Güemes M, Rahman SA, Hussain K. What is a normal blood glucose? *Arch Dis Child* (2016) 101:569–74. doi: 10.1136/archdischild-2015-308336

31. Harris DL, Weston PJ, Harding JE. Feeding patterns of healthy term newborns in the first 5 days-the glucose in well babies study (GLOW). *J Hum Lact* (2022) 38:661–9. doi: 10.1177/08903344221087605

32. 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) 104:4356–64. doi: 10.1210/jc.2018-02763

33. DuBose SN, Kanapka LG, Bradfield B, Sooy M, Beck RW, Steck AK. Continuous glucose monitoring profiles in healthy, nondiabetic young children. *J Endocr Soc* (2022) 6:bvac060. doi: 10.1210/jendso/bvac060

34. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group, Fox LA, Beck RW, Xing D. Variation of interstitial glucose measurements assessed by continuous glucose monitors in healthy, nondiabetic individuals. *Diabetes Care* (2010) 33:1297–9. doi: 10.2337/dc09-1971

35. De Angelis LC, Brigati G, Polleri G, Malova M, Parodi A, Minghetti D, et al. Neonatal hypoglycemia and brain vulnerability. *Front Endocrinol (Lausanne)* (2021) 12:634305. doi: 10.3389/fendo.2021.634305

36. Fong CY, Harvey AS. Variable outcome for epilepsy after neonatal hypoglycaemia. *Dev Med Child Neurol* (2014) 56:1093–9. doi: 10.1111/dmcn.12496

37. Ludwig A, Enke S, Heindorf J, Empting S, Meissner T, Mohnike K. Formal neurocognitive testing in 60 patients with congenital hyperinsulinism. *Horm Res Paediatr* (2018) 89:1–6. doi: 10.1159/000481774

38. Worth C, Tropeano Y, Gokul PR, Cosgrove KE, Salomon-Estebanez M, Senniappan S, et al. Insight into hypoglycemia frequency in congenital hyperinsulinism: evaluation of a large UK CGM dataset. *BMJ Open Diabetes Res Care* (2022) 10(3):e002849. doi: 10.1136/bmjdrc-2022-002849

39. 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) 44:1136–50. doi: 10.1002/jimd.12383

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Standardised practices in the networked management of congenital hyperinsulinism: a UK national collaborative consensus

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Congenital hyperinsulinism (CHI) is a condition characterised by severe and recurrent hypoglycaemia in infants and young children caused by inappropriate insulin over-secretion. CHI is of heterogeneous aetiology with a significant genetic component and is often unresponsive to standard medical therapy options. The treatment of CHI can be multifaceted and complex, requiring multidisciplinary input. It is important to manage hypoglycaemia in CHI promptly as the risk of long-term neurodisability arising from neuroglycopaenia is high. The UK CHI consensus on the practice and management of CHI was developed to optimise and harmonise clinical

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management of patients in centres specialising in CHI as well as in non-specialist centres engaged in collaborative, networked models of care. Using current best practice and a consensus approach, it provides guidance and practical advice in the domains of diagnosis, clinical assessment and treatment to mitigate hypoglycaemia risk and improve long term outcomes for health and well-being.

KEYWORDS

congenital hyperinsulinism, glucose, hypoglycaemia, consensus, patient organisation, standardised practice, treatment

Introduction

Congenital hyperinsulinism (CHI) is a rare condition characteristed by severe and often refractory hypoglycaemia in infants and young children, due to inappropriate excess insulin secretion from pancreatic β-cells (1). CHI has heterogeneous aetiology, but the majority are due to different underlying genetic variants (2) most commonly in the ATP sensitive K^+ channels coupling glucose levels and insulin secretion.

Estimates of prevalence of CHI vary from 1 in 50, 000 to 1 in 2, 500, the latter in populations with high consanguinity rates (3–6). In the UK, the minimal incidence of CHI measured by genetic testing referral rates for hyperinsulinism persisting beyond 6 months of age, is 1 in 28, 389 live births (7). CHI represents a significant clinical burden, with an estimated annual cost of illness to the UK National Health Service of approximately £3, 408, 398 (8), not counting long-term costs from neurodisability.

CHI commonly presents in the neonatal period, with initial investigations and management initiated by general paediatric or neonatal teams. While a number of published reviews have summarised broad management principles and strategies and an international consensus guideline is due for publication, there is no clinical practice collaborative consensus that underpins the shared network treatment of CHI (9–11). This group has aimed to collaborate and harmonise current best practice to produce a working guideline for the management of CHI in the UK, with a focus on practical aspects including diet, medication changes and monitoring. This guideline is intended for use by general paediatricians, neonatologists, paediatric endocrinologists, surgeons, specialist nurses, dietitians and speech and language therapists, as well as GPs, involved in the day-to-day management of CHI patients through an effective network of care.

Methods

A consensus group was derived from healthcare professionals with extensive experience in the management of CHI in centres across the UK (comprising Northern Ireland, Wales, Scotland and England) forming the CHI Special Interest Group (SIG), a subcommittee of the British Society for Paediatric Endocrinology and Diabetes (BSPED). The group also included patient representatives from the UK CHI Charity, Children's Hyperinsulinism Charity (CHC). Members of the group were tasked with writing components for discussion. The group met virtually on 8 occasions over a period of 18 months at regular intervals (ending March 2023) to discuss available evidence and experience, with additional separate smaller group meetings. The highest level of evidence from the current scientific literature was used as the basis of recommendations agreed by the entire group. The GRADE criteria was not adopted by the group on grounds of paucity of high quality evidence in formulating practice advice in a networked model and the concomitant development of an international CHI guidance providing general recommendations. Where evidence was deemed insufficient or inadequate to be able to make a definitive recommendation, the authors discussed a consensus of centre experiences to frame a working recommendation appropriate for the network. Consensus was achieved by presentation of evidence in published literature, followed by discussion among group members and subsequent voting to determine a significant majority (>80%).

The terminology to describe hypoglycaemia from hyperinsulinism is variable and can include Congenital Hyperinsulinism (CHI), Hyperinsulinaemic Hypoglycaemia (HH), Hyperinsulinism (HI). For the purposes of this guideline, CHI will be adopted as the preferred term regardless of genetic aetiology or the age of onset of illness.

The specific areas in CHI, covered by the consensus are:

- Presentation and diagnosis;
- Management options including fluids, feeds, medications and surgery;
- Considerations for discharge from hospital;
- The role of the extended multidisciplinary team (MDT);
- Long-term management and outcomes;
- Potential future therapies.

Presentation and Diagnosis

Hypoglycaemia: diagnostic and therapeutic thresholds

Hypoglycaemia occurs when blood glucose is low, ie less than the normal range (4.0 and 6.0 mmol/L) (12). As patients with CHI

have greater risk of neuronal injury and neurodevelopmental sequelae due to severe hypoglycaemia and the absence of ketone production, it is pragmatic to keep glucose levels close to the normal cut-off of 4.0 mmol/L as possible rather than risk possible neuronal injury with lower levels (9).

The definition of neonatal hypoglycaemia itself is controversial. Studies suggest using a cut off glucose of 2.0 mmol/L in asymptomatic neonates without risk factors for CHI, such as fetal distress and intra-uterine growth restriction, in contrast to more traditional levels of 2.6 mmol/L (13). However, there is some evidence of the risk of long-term executive motor function and visual motor function impairment at levels of both 2.0 and 2.6 mmol/L, albeit without correlation to school performance (14, 15), implying the absence of an agreed safe cut off glucose level. In view of the uncertainties over an agreed hypoglycaemia threshold, it is prudent to aim for a glucose level at or near the normal level.

Glucose threshold for investigation – 3.0 mmol/L

For operational purposes, it is important to define a cut off glucose level to commence (16) investigations and treatment for hypoglycaemia. An international consensus defined this as 3.3 mmol/L (17); however, this level may be too high and could lead to over-investigation and the finding of detectable insulin levels in many neonates without CHI, with consequent over diagnosis of CHI. According to the experience of the specialist centres a level of 3.0 mmol/L is a reasonable compromise between 3.3 mmol/L and the lower traditional threshold of 2.6 mmol/L and could be used as a practical threshold, particularly in the presence of severe hypoglycaemia associated with a high glucose infusion rate (GIR).

The timing of hypoglycaemia screening is complicated by transitional hypoglycaemia in the first 48-72 hours of life when neonates adapt to extra-uterine conditions. Guidelines have been developed by the British Association for Perinatal Medicine (BAPM) for the management of neonatal hypoglycaemia, although it is important to remember these are for generic neonatal hypoglycaemia and not specifically for CHI 18). Current international recommendations suggest investigation for CHI beyond this period (17). However, severe and/or recurrent hypoglycaemia, undetectable blood glucose levels at any time, or two or more episodes <3.0 mmol/L with glucose infusion rate (GIR) >8 mg/kg/min within the first 48 hours of life, should alert the clinician to the potential diagnosis of CHI even within this period. A high GIR beyond 48 hours after birth in the context of severe and recurrent hypoglycaemia is a a strong pointer to CHI over transitional hypoglycamia or metabolic causes of hypoglycaemia (19).

GIR, a practical measure of glucose requirement, can be calculated from the rate of infusion, concentration of dextrose and body weight [mg/kg/min) = (Fluid rate (ml/hr) X % Dextrose)/(Weight (kg) X 6)] or by using online calculators (20, 21). A normal GIR in the neonatal period is 4-6 mg/kg/min; rates exceeding 8 mg/kg/min suggest the diagnosis of CHI (9, 17).

The biochemical diagnosis of hyperinsulinism relies on the finding of a detectable insulin level at hypoglycaemia. Insulin has a short half life of around 6 minutes (9) and therefore may be missed if hypoglycaemia blood screening samples are delayed. If insulin is undetectable but clinical features point to CHI, further diagnostic samples may be required. Excess insulin secretion suppresses ketones in addition to producing hypoglycaemia and may be used as a biochemical surrogate for hyperinsulinism. Therefore, the combination of hypoglycaemia (at or below 3.0 mmol/L), suppressed ketones and fatty acids as well as an increased GIR is diagnostic of CHI.

In the absence of measurable insulin levels during hypoglycaemia or a high GIR, an alternative cause should be pursued in discussion with the regional endocrine and/or designated CHI specialist centre. The possibility of an underlying metabolic disorder should also be considered, particularly if serum ammonia levels are raised or if there is lactic acidosis. Hypopituitarism and adrenal insufficiency may also need to be excluded as causes of hypoglycaemia.

Glucose threshold for treatment – 3.5 mmol/L

It is aspirational to aim for glucose > 4.0 mmol/L in the treatment of hypoglycaemia due to CHI; however, this may require significant escalation of treatment (for example greater concentrations of dextrose) that may lead to therapeutic side effects. The risk of overtreatment has to be balanced against the risk of neurological injury at lower glucose levels (9); by consensus a practical therapeutic glucose threshold level of ≥3.5 mmol/L was agreed.

Initial investigations

A "hypo screen sample" should be obtained at the time of spontaneous hypoglycaemia and before treatment with glucose (Table 1). Samples obtained after glucose treatment may misrepresent insulin, free fatty acids (FFA) and beta hydroxybutyrate (BOHB) levels at the time of hypoglycaemia, thereby delaying the diagnosis of CHI. If insulin samples cannot be obtained at the time of hypoglycaemia and the diagnosis of CHI is doubtful, hypoglycaemia may need to be induced with careful glucose monitoring to reach a diagnosis after discussion with the regional endocrine/CHI centre. Pre-prepared "hypopacks" which contain blood sample containers (Table 1) can be helpful. In cases of difficult sample collection or small blood volumes, priority should be given to plasma glucose and serum insulin for analysis. Plasma glucose samples should always be taken peripherally to avoid any contamination from intravenous glucose that could cause false interpretation of results. It is important to ensure that insulin samples are analysed with a rapid turnaround time (expected 2-3 working days) to avoid delays in establishing the diagnosis of CHI. Most modern assays now detect insulin at low concentrations. However, with highly sensitive insulin assays (22), there is potential for overdiagnosis of CHI. As laboratory analyses vary, assay-dependent insulin cut-off levels (and other analytes such as BOHB and FFA) should be determined for clinical significance; in

TABLE 1 Initial hypo screen investigations (those in bold indicate a higher priority).

Samples taken at the time of hypoglycaemia	May be taken after hypoglycaemia correction	First urine after hypoglycaemia
- **Glucose,** lactate - **Insulin, C-peptide** - FFA, BOHB - Cortisol * - GH *	- Acylcarnitine - Plasma amino acids - Ammonia - Electrolytes - Liver function	- Urine organic acids

FFA, free fatty acids; BOHB, beta hydroxy butyrate; GH, growth hormone.
To check specimen/tube/volumes with local laboratory.
*GH and cortisol axis may need to be evaluated to exclude hypopituitarism.

most cases insulin levels above 12 pmol/L (2 mU/L) indicate meaningful hyperinsulinism. In case of doubt about the diagnosis of CHI, a discussion with the regional endocrine and/or CHI centre is advised.

The level of insulin at the time of hypoglycaemia does not correlate with the severity of the disease. Insulin may be undetectable in up to 20% of cases (23). Therefore in the presence of clinical features of CHI, the diagnosis may hinge on the evidence of insulin action, i.e., low ketones/BOHB and low free FFA. Point of care testing for BOHB can be useful to demonstrate low ketone production, particularly in older children. In neonates on dextrose infusions or frequent feeds, BOHB is less reliable but should be used if facilities are available. However, the presence of modest ketones is not sufficient to exclude CHI (22).

C-peptide can also aid the diagnosis, particularly where hyperinsulinism is suspected but not proven. A level ≥ 0.5 ng/mL (>165.5 pmol/L) may offer a high sensitivity of a CHI diagnosis (23). However, as C-peptide has a longer life than insulin, it may represent secretion prior to hypoglycaemia. Further, lower levels of detection are likely to be assay dependent; therefore, C-peptide cut-offs to indicate high probability of CHI may vary between centres.

The possibility of exogenous insulin administration as a cause for hypoglycaemia should be considered if C-peptide levels are undetectable but insulin is present at hypoglycaemia. If factitious and fabricated illness (FII) is suspected, samples should be obtained specifically for insulin analogues. It is important to highlight that not all insulin assays are able to detect insulin analogues and some assays only detect certain insulin analogues, but not others. If the diagnosis of CHI is in doubt or if FII is considered by the clinical team, the regional endocrine and/or CHI centre should be contacted.

UK referral pathways and CHI specialist centres

NHS England commissions a Highly Specialised Congenital Hyperinsulinism (CHI) service in England. CHI patients in Wales and Northern Ireland can also be referred to these centres through separate arrangements. The regional Scottish endocrine centres manage patients with CHI locally in Scotland in collaboration with the English CHI centres.

The CHI centres have established advice and referral networks which include tertiary paediatric endocrinology centres and district general hospitals (DGH). It is expected that the diagnosis and early treatment of hypoglycaemia in neonates and infants is considered in local units before proceeding with a referral to a CHI centre. If CHI is unlikely, the patient should be managed locally. In cases of uncertainty of diagnosis, the networked CHI centre should be consulted. If the cause of hypoglycaemia is likely to be of metabolic aetiology, the regional centre for inherited errors of metabolism should be contacted. In some cases, it is not clear if hypoglycaemia is due to CHI or metabolic aetiology; in such cases both CHI and metabolic opinions should be sought.

The model referral pathway for CHI is shown in Figure 1. The regional endocrine centre should consider liaision with the CHI centre soon after diagnosis of CHI to discuss investigation and treatment options and if referral to a CHI centre might be required.

The CHI centre should communicate effectively with the local hospital to discuss efficacy of treatment, safety issues and follow up plans in a network care plan. If a patient responds to diazoxide in a dose less than 7 mg/kg/day without side effects, as per consensus, care could be retained locally, with update on progress at regular intervals until hospital discharge. If the dose of diazoxide is increased to or beyond 7 mg/kg/day due to insufficient response, unacceptable side effects, or persistent requirement for intravenous dextrose, the local hospital should discuss with the CHI centre for potential transfer. The CHI centre should assume responsibility for the decision to transfer based on individual circumstances. If a CHI patient shows signs of resistance to diazoxide, second line treatment with a synthetic somatostatin analogue such as octreotide may be required. In most cases, octreotide should be commenced and managed in a CHI centre. Additionally, the CHI centre should be involved if surgical central venous access or pancreatic surgery is envisaged. It is accepted that the model (DGH-Regional Endocrine Centre-CHI centre) may not apply to all hospital centres depending on local expertise, preferences and arrangements and may require local adaptation for patient need. Nonetheless, the principles of efficient networked care remain the same, allowing for variations in the model and potential applications in countries outside the UK.

Acute and immediate management

The immediate treatment goal in CHI is to achieve a safe glucose level to prevent the risk of hypoglycaemia-induced brain injury. Hypoglycaemia should be treated promptly, once

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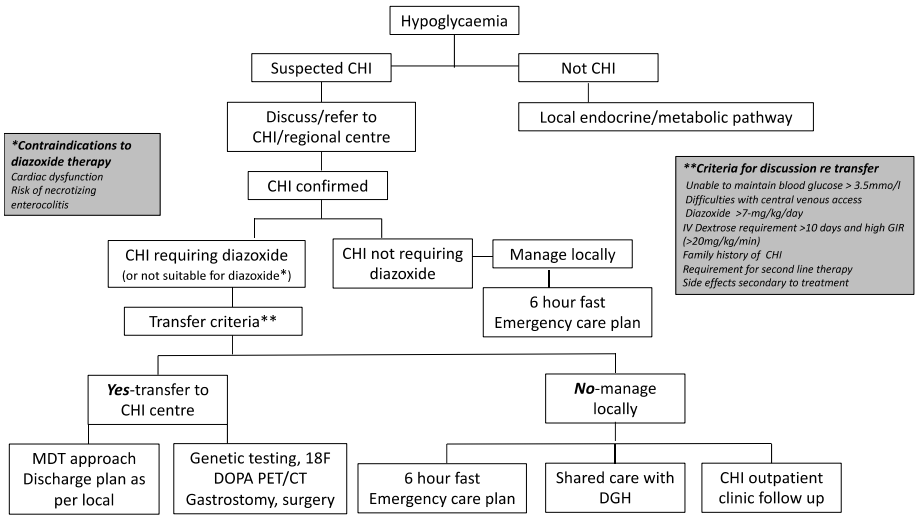

FIGURE 1
Referral pathways and criteria for congenital hyperinsulinism diagnosis and treatment in a networked model of care. CHI, congenital hyperinsulinism; DGH, district general hospital.

hypoglycaemia screening bloods have been taken. A bolus of 2.5mls/kg of 10% glucose is recommended (24) to treat acute hypoglycaemia followed by a continuous glucose infusion to prevent rebound hypoglycaemia. Subsequent smaller bolus doses of 1-2mls/kg of 10% glucose (if required) are suggested in CHI patients to avoid rebound hypoglycaemia.

In most cases, oral or nasogastric feeds, even with increased carbohydrate content, are not enough to attain normoglycaemia and intravenous access is required. As GIR is high in CHI, failure to correct hypoglycaemia with 10% glucose at a standard maintenance rate, makes CHI likely. A 10% glucose infusion of 5 mL/kg/hr (or 120 mL/kg/day) equates to a GIR of 8.3mg/kg/min and if hypoglycaemia persists with this GIR, the possibility of hyperinsulinism should be investigated. Until biochemical diagnosis of hyperinsulinism is confirmed, initial glycaemic stabilisation should be achieved with a high concentration glucose infusion (15-20%) administered by a central venous catheter. In addition, glucagon administration by intravenous/subcutaneous infusion is useful for initial glucose stabilisation, reduction of the total fluid requirement and a reduction in the potential risk of fluid overload associated with diazoxide treatment (25). At present, continuous subcutaneous glucagon infusions are not available for home use, due to instability of glucagon susceptible to fibrillation and precipitation in aqueous solutions. Soluble glucagon analogues have been successfully investigated in clinical trials and may become available for use in the near future (26).

Following initial glycaemic stabilisation, definitive treatment of CHI should be considered with a view to safe discharge from the hospital. Treatment with diazoxide is the first line of definitive clinical management and is pivotal to a successive clinical decision pathway outlined in Figure 2. Diazoxide responsiveness is a key criteria that determines requirement for CHI specialist care, genetic investigations and second line therapy with octreotide. An escalating diazoxide dose > 7 mg/kg/day (indicating partial response) (9), consolidated by consensus, was deemed unsuitable for local care and more appropriate for CHI specialist care. Patients requiring higher doses of diazoxide or those unresponsive to diazoxide have a higher propensity for monogenic aetiology (9) requiring genetic investigations utilising a targeted gene panel to screen for disease-causing variants. In patients with early indication of failure of medical therapies and high GIR, rapid analysis of the *ABCC8* and *KCNJ11* genes is required to expedite the potential diagnosis of focal CHI. Discussion with the local CHI centre regarding the urgency and type of genetic testing is advised, especially if there is a family history of diabetes and large birth weight infants.

Medication

Glucagon

Glucagon increases endogenous glucose production by stimulating glycogenolysis, gluconeogenesis and lipolysis, which is typically inhibited in the presence of insulin excess. It can be used as an intramuscular injection in an emergency or as a continuous intravenous or subcutaneous infusion. The starting dose of glucagon is 2.5-5.0 micrograms/kg/hour, with dose escalation in steps of 2.5 mcg/kg/hr to maintain glucose levels above 3.5 mmol/L (Table 2). The maximum dose of glucagon generally used is 20 mcg/kg/hr (25, 27). To prepare a continuous infusion, glucagon formulation for intramuscular injection can be diluted in 0.9% sodium chloride or 5% dextrose. Glucagon solution should be changed every 12-24 hours to avoid catheter occlusions due to glucagon instability and precipitation in prepared solutions (28). It is preferable not to use line filters to minimise the risk of line occlusion. Glucagon can be administered peripherally or via central catheters with clear fluids, but avoided with parenteral nutrition or high concentration of dextrose (>15%), as the solute load might aggravate precipitation. The use of additional clear fluids down the same line as glucagon infusion can help to reduce precipitation and improve delivery of the active drug.

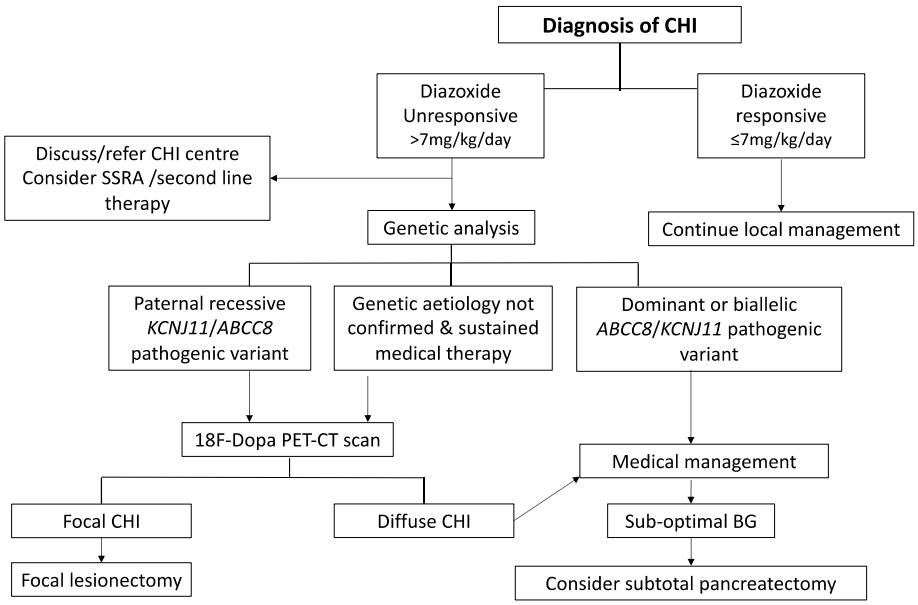

FIGURE 2
Clinical pathway of diagnosis and management for patients with congenital hyperinsulinism (CHI). Clinical decisions hinge on criteria such as diazoxide responsiveness, genetic investigations, 18 Fluoro Dopa PET-CT/MR imaging and response to medical therapy.

Glucagon is helpful to reduce the fluid requirement in neonates predisposed to fluid overload and in those at risk of developing pulmonary hypertension following treatment with diazoxide (29). From a glycaemic perspective, glucagon treatment is safe and effective in reducing GIR (25, 27). Glucagon associated side effects are infrequent; however it is important to recognise necrolytic migratory erythema (NME), a rare but severe form of rash associated with high doses over prolonged periods and disappearing after discontinuation (9). Prolonged glucagon may also cause vomiting, weight loss and coagulopathy. For the latter, particularly in those requiring high concentration (≥15%) glucose, prophylactic anticoagulation with enoxaparin should be considered to avoid central venous catheter-related thrombosis (30).

Diazoxide

Following an established diagnosis of CHI, treatment with oral diazoxide should be started as first line treatment if there is no spontaneous resolution of hypoglycaemia. Diazoxide, an oral nondiuretic benzothiadiazine, acts on the sulphonylurea receptor sub-unit of the ATP-sensitive potassium (KATP) channel to keep the channel in the open conformation leading to β-cell membrane stabilisation. This in turn, causes a reduction in calcium influx into the β-cell thereby reducing calcium mediated insulin efflux. Whilst diazoxide is effective in most individuals with non-KATP channel CHI, the majority of patients with a KATP channel mutation show poor response to the drug, although those with mild dominantly acting KATP channel mutations may respond well to diazoxide. There are also rare reports of infants with biallelic pathogenic

TABLE 2 Drug dosing and monitoring.

Drug	Dose	Pre initiation	Post initiation	Monitoring	Side effects/risks
Diazoxide	Initial 2-5mg/kg/d in 2-3 divided doses Increased to 20mg/kg/d	Fluid restriction 130-150 ml/kg/d- for at least 24-48 hrs prior to starting ECHO	ECHO- 10-14 days later*	UEs, urate, FBC,- 4-6 monthly	Fluid retention, Pulmonary hypertension, NEC, neutropenia, thrombocytopenia, hypertrichosis
Chlorothiazide	Initial 7mg/kg/d in 2 divided doses			UEs	Hyponatremia
Octreotide*	5-30mcg/kg/day, usually given 6-8hrly by sc injection	Abdo US, LFTs		LFTs, TFTs Growth, Abdo US- annually	NEC, biliary sludge, growth faltering, GI disturbance, cholelithiasis, pituitary hormone suppression
Glucagon (1000mcg/ml)	5-20mcg/kg/hr (start at 2.5-5.0 mcg/kg/hr)			Infusion set to be changed every 12 hours	IV line occlusion, NME

* especially if clinical concerns/existing cardiac issues. FBC, full blood count; GI, gastrointestinal; IV, intravenous; LFTs, liver function tests; NEC, necrotizing enterolitis; NME, necrolytic migratory erythema; TFTs, thyroid function tests; sc, subcutaneous; UEs, urea and electrolytes; US, ultrasound.

variants in *ABCC8* being diazoxide responsive and a few patients with known pathogenic variants in *ABCC8* may have a partial response (31–33) although satisfactory glycaemic stability is not always achieved.

Diazoxide related side effects have been described in observational studies (34) with 10% patients expected to show minor or major concerns. In the early stages of use, diazoxide therapy often exacerbates fluid retention, necessitating the use of a diuretic such as chlorothiazide. Pulmonary hypertension can occur in 2-7% patients, following diazoxide therapy, arguably more so in those with pre-existing cardiovascular abnormalities. Therefore, an echocardiogram (ECHO) to assess cardiac structure and pulmonary artery pressures before diazoxide is recommended. A repeat ECHO can be considered 10-14 days after starting diazoxide treatment, especially if there are clinical concerns (29, 34–36). In cases where an ECHO cannot be easily obtained, close monitoring for cardiovascular compromise should be ensured until a cardiac review is available. In infants with previous but resolved pulmonary hypertension, diazoxide should be used with caution.

Treatment with diazoxide mandates a fluid volume restriction, including feeds and intravenous infusions to total of 130-150ml/kg/day in term neonates for 24-48 hours prior to diazoxide therapy to reduce the risk of pulmonary hypertension. In premature babies with higher risk factors for pulmonary hypertension, fluid volume may need to be restricted further to 120ml/kg/day. Volume reduction may be achieved by high glucose concentrations delivered by central venous catheters and/or by the use of glucagon to reduce glucose dependence.

Following diazoxide use, fluid volumes may be liberalised gradually in the absence of pulmonary hypertension, although a total fluid volume of >150ml/kg/d should be generally avoided in the first 2 weeks of life.

A starting diazoxide dose of 2.0-5.0 mg/kg/day in 2-3 divided doses is suggested by consensus, together with a chlorthiazide dose of 7mg/kg/day in 2 divided doses (Table 2). The lower dose of diazoxide may be considered appropriate in neonates likely to be diazoxide responsive and sensitive, for instance in those where the hypoglcaemia is due to perinatal stress induced hyperinsulinism, including intra uterine growth restriction and being small for gestational age (37, 38).

If pulmonary hypertension is suspected, diazoxide therapy should be discontinued immediately, as dose reduction is not effective in mitigation. Instead, further fluid restriction may be required with the need for high concentration glucose and/or glucagon to reduce the risk of hypoglycaemia. Additionally, an urgent cardiac opinion should be sought for confirmation of pulmonary hypertension.

Diazoxide use can be associated with other problems, including necrotising enterocolitis (NEC) (39, 40), more so in those with predispositions such as prematurity (40). Other side effects include neutropenia and thrombocytopenia in upto 9% of patients (10, 36) prompting the need to check blood counts both at initiation and in follow up. If neutropenia and/or thrombocytopaenia are present prior to treatment, diazoxide should be used with caution with regular monitoring. Rarer side effects include pericardial effusion requiring surgical drainage and paradoxical hypoglycaemia with high dosage (41, 42). In the long term, it is common to observe excess hair growth with doses > 5mg/kg/day (36). Table 1 considers the dosage, monitoring guide and side effects of diazoxide and other drugs in CHI.

In view of the proclivity to side effects, it is advisable to start diazoxide in a lower dose, escalating upwards to a maximum of 20mg/kg/day, depending on the response. A dose in excess of 15 mg/kg/day is rarely effective and indicates diazoxide unresponsiveness. The majority of patients with biallelic pathogenic variants in *ABCC8* and *KCNJ11* are diazoxide unresponsive, necessitating second line treatment. In such cases, diazoxide can be stopped abruptly without gradual de-escalation. Further, it is advisable to avoid combination therapies in CHI to reduce the risk of therapy related major side effects.

Following a satisfactory glyaemic response to diazoxide, an age appropriate fast should be undertaken, prior to discharge from the hospital (discussed in a following section).

In the longer term, monitoring of growth, renal function, urate/uric acid and blood count is recommended. It is not necessary to increase the dose of diazoxide progressively with gain in weight, if glucose levels remain stable. In such cases the therapeutic strategy would be to "out grow the dose", allowing for a gradual reduction in the dose for body weight. In those patients on previously higher doses of diazoxide (>5mg/kg/day), with relative dose reduction to <2-3mg/kg/day, as agreed by consensus, active reduction could be considered. Diazoxide dose reduction can be managed safely at home under supervision by the treatment team. These could include frequent virtual consultations by specialist nurses with prescribing authorisation. However, if stopping or reducing diazoxide therapy poses potential risks, inpatient admission for monitoring may be required. For those successful in active home withdrawal of diazoxide, a safety fast (age appropriate) and hospital admission is generally recommended; this can be done following discussion with the clinical team.

In all cases, initiation of diazoxide treatment should be accompanied by diuretic use. However, fluid retention becomes less problematic beyond the age of one year and chlorthiazide can be safely discontinued, especially if the diazoxide dose remains small (< 5 mg/kg/day) and does not require escalation.

Octreotide

Synthetic Somatostatin Analogues (SSA) such as Octreotide are used as second line therapy for children with CHI who are not responsive to diazoxide (43) (Figure 2). SSAs act through somatostatin receptors to inhibit cAMP mediated insulin secretion. Octreotide, a short acting SSA, is administered either as 6–8 hourly subcutaneous injections or by continuous intravenous or subcutaneous infusion.

The starting dose of octreotide is usually 5 micrograms/kg/day, which should only be initiated in a CHI specialist centre or regional centre with expertise/experience (Table 2). Initial administration of octreotide often leads to hyperglycaemia, but the effect is not sustained. Loss of initial effect or tachyphylaxis sets in after 48 hours when dose increments are required to prevent hypoglycaemia. The dose of octreotide can be increased in 2.5 microgram/kg/day steps to a maximum of 30 microgram/kg/day.

Beyond this dose, side effects become apparent, with only marginal improvement in efficacy.

Octreotide may be administered via a continuous subcutaneous infusion using an insulin pump (44). The responsibility for initiating and advising on continuous octreotide infusions lies with the CHI centre with special consideration given to variable drug concentrations, ensuring correct conversion of micrograms to insulin units and anticipation of pump malfunction.

Octreotide acts on many tissues and has an impact on the hepatobiliary system with reports of hepatitis, biliary sludging and gall stones (43). Infants treated with octreotide have been reported to develop NEC, probably as a result of reduced splanchnic blood flow (45–47). Octreotide potentially reduces pituitary growth hormone production; while short stature is not commonly recognised, this may also be under-reported (48).

An alternative to short acting octreotide is the use of long acting SSA like octreotide LAR (e.g. Sandostatin LAR™ or Olatuton™) and somatuline autogel (e.g. Lanreotide™) (49–52). These can be considered in infants over 6 months of age and offer the prospect of once monthly subcutaneous or intramuscular injections, thereby reducing the need for multiple daily injections or cumbersome pump therapy. The efficacy profile seems satisfactory although studies remain observational without a control arm to judge true benefit over harm. Further, the use of long acting SSA is complicated by similar side effects as octreotide with the additional disadvantage of the persistence of side effects due to long interval dosing.

Alternative SSAs such as pasireotide have been used in difficult cases but efficacy remains contentious (53). It is not recommended to use pasireotide or other repurposed medications unless within a research capacity.

Feeds

Management of feeds is integral to the clinical management strategy in patients with CHI. Infants may require carbohydrate supplements alongside medications to facilitate weaning from intravenous dextrose to enteral feeds. Additional carbohydrates can be provided in the form of glucose polymers, high energy formula, pre-term formulas or breast milk fortifiers. It is recommended that infants requiring additional oral carbohydrate to prevent hypoglycaemia should be referred to an experienced paediatric or neonatal dietitian.

Addition of a glucose polymer should be started in a low dose at 1 - 2.5% (Appendix 1). An increase can be considered after 48 hours if no signs of intolerance are noted, such as vomiting or loose stools. Further increments can be made every 48 hours by 1 – 2%, up to a maximum of 5%, i.e. not exceeding 12.5% total carbohydrate including the carbohydrate content already provided in milk feeds. Examples of some infant milks and supplements for infants with CHI are shown in Appendix 1.

In neonates at increased risk of developing gut ischaemia such as **NEC**, extreme caution should be exercised in the use of supplemental carbohydrates that increase solute osmolar load. Only following discussion and assessment by an experienced paediatric/neonatal dietitian should a glucose polymer be added in such cases.

Ongoing dietetic supervision is required as the protein to energy ratio often becomes distorted with carbohydrates supplementation, with a consequential negative effect on growth in the infant (54). To achieve adequate growth, the protein to energy ratio should be maintained between 7.5% and 12%. This could be achieved by using a higher energy infant formula (which also has a higher protein content), or concentrating a standard infant formula along with a glucose polymer for term infants. Fortifier may be added to expressed breast milk or a formula for preterm infants can be used if breast milk is not available.

Where infants are managed outside of CHI specialist centres, the addition of a glucose polymer may be more challenging as appropriate scoops or specialist formula may not be readily available. In the absence of a specialized CHI dietitian, a paediatric tertiary centre dietitian or neonatal dietitian should be contacted to provide appropriate support.

Breastfeeding

If a mother wishes to breastfeed, support should be provided to express regularly to protect her milk supply, and to allow her breastmilk to be given as expressed breast milk (EBM). The duration of EBM use is expected to be moderately short and likely to be dependent on diazoxide dose, response and individual circumstances. If fluid intake is restricted, it may still be possible to put the infant to the breast for short periods which can help with bonding and maintain milk supply but requires agreement with the treatment team. Advice should be sought from specialist staff in this area such as breastfeeding advisors to increase the likelihood of success. Additional carbohydrate in the form of glucose polymers can be added to EBM if blood glucose levels are not maintained by medication alone.

Breast milk is naturally low in protein; the use of breastmilk fortifiers for pre-term infants should be considered over the simple addition of glucose polymers to ensure maintenance of the protein to energy ratio. Breast milk fortifiers are not prescribable in the community and therefore, if needed a regular hospital supply following discharge home may be required. This will need to be agreed with the wider community team. Alternatively, a protein supplement such as Protifar™ may be used to ensure adequate protein intake.

Continuous feeding

Some infants require feeding at least every 3 hours, if not more regularly, both day and night to ensure glycaemic stability. However, this can become exhausting and unsustainable for the parents. In such cases continuous feeds through a nasogastric or gastrostomy tube may be required.

If an infant is to be discharged home on pump feeding, a gastrostomy tube is recommended over a nasogastric tube to prevent aspiration of the feed. Additional advice on safety should be provided to the parents, e.g., how to prevent the risk of the tube becoming entangled around the neck at night. It may be important for parents to be in proximity to the child at night to hear the pump alarm in the event of a pump occlusion. To mitigate the risk of pump failure, a real-time continuous glucose monitor (CGM), with

an alarm to detect hypoglycaemia, could be considered. Additionally, in the event of pump failure and subsequent risk of hypooglycaemia, the patient must be provided with a spare feeding pump to ensure that continuous feeding can be maintained.

Slow release carbohydrates

Older children may require additional glycaemic stability through the addition of slow release carbohydrates such as uncooked corn starch (55). Such supplements are preferably used in children older than 2 years with intestinal disaccharidases capable of breaking down carbohydrate in the form of starch. Corn starch, mixed in fluids at room temperature, may be commenced at 0.5g/kg/dose and increased gradually to a maximum of 2g/kg/dose to optimize therapeutic benefit while preventing side effects such as abdominal pain and loose stools. Corn starch is generally given with a pre-bedtime feed or snack but can be used up to 3-4 times a day if indicated and tolerated.

Weaning

Introduction to solid foods should be commenced when the infant displays normal feeding cues, has good head control, and be no later than 6 months for term infants. For preterm infants, the local neonatal team/dietitian should be consulted for advice on progression to solid foods. Normal healthy weaning advice is recommended using complex carbohydrate foods with avoidance of simple sugars in the early stages until the infant is on a mixed diet. High sugar purees are absorbed rapidly with resultant hypoglycaemia but with potential for post prandial hypoglyaemia and therefore best avoided, except for the treatment of acute hypoglycaemia.

Growth monitoring

Careful monitoring of weight and overall growth should be a priority for all patients so that feeds can be adjusted to optimize growth. For infants on diazoxide, fluid volumes are restricted with the potential to constrain growth. Thus, dietetic overview is essential through liaison with community staff e.g., health visitors to ensure that the infant receives appropriate nutritional advice. Regular weighing will ensure that feed volumes can be adjusted according to weight change to optimise growth.

Clear written instructions should be given when additional glucose polymers are added to feeds to ensure that the correct percentage is chosen. In some instances, weighing feed ingredients with scales, rather than the use of scoops, may be preferable for simplicity of formulation and accuracy of constituents (56).

Feeding problems

A proportion of patients with CHI have significant challenges with oral feeding (57, 58) requiring support from a Speech and Language Therapist (SLT) at an early stage. Feeding problems can occur early precluding establishment of milk feeding and successful progression with weaning and textures. Long term tube feeding can impair oral feeding progress with the development of oral aversion. The exact cause of feeding problems is not known but is most likely multifactorial, with complex entangled interplay of therapeutic, environmental, developmental and psychosocial issues (16, 59). The management of feeding problems in CHI requires early recognition and timely multidisciplinary involvement to mitigate such factors.

The use of anti-reflux medication such as proton pump inhibitors and thickened feeds may reduce vomiting and therefore address a few components in the range of causative factors associated with feeding problems. Parents should receive consistent support from SLT to allay frustration and feed related anxiety. SLT should also aim to support other caregivers, including nursing staff who often actively feed infants in the hospital and raise awareness of cue-based feeding practices to minimise the risk of worsening of feeding problems. While feeding problems are common in CHI patients, these should not preclude or dissuade oral feeding. The treatment team incorporating a SLT, should have a proactive aim to allow at least partial oral feeding, appropriate to the child's age and developmental level. This remains relevant when beginning weaning and progressing through textures. At all stages in managing feeding difficulties, it is important to be responsive to individual acceptance of oral intake. Pushing oral feeding at an early stage in the presence of stress cues will adversely result in later food refusal that might be more challenging.

In some infants, feeding problems are complicated by intolerance to cow's milk protein which may improve by changing to a more appropriate feed. If changing feeds, glucose levels should be monitored to ensure clinical stability during the transition period.

Intercurrent illness/surgery advice

Those infants who are on medication and/or additional carbohydrates should be provided with an intercurrent illness plan (emergency regime) which includes instructions on the use of a glucose polymer at a percentage suitable for the age of the child (Appendices 2, 3). Parents should be given written instructions on when and how to use emergency regimens. Adequate supplies must be maintained, especially for periods when they are away from the home such as during holidays. The emergency feeds should be tried when the child is well to ensure it will be taken when needed during illness episodes. Glucose polymer drinks can be flavoured with squash or cordial, if preferred, to improve palatability. During severe diarrhoeal/vomitting illnesses medical advice should be sought and hospital admission may need to be considered. In all cases of supplementation with glucose polymers, good oral hygiene should be maintained and regular dental review advised to minimize the potential for dental caries from excess sugar intake. Prolonged periods of fasting should be avoided, including those for procedures needing general anaesthesia. Administration of intravenous fluids with glucose with regular monitoring during the procedure is recommended to avoid hypoglycaemia. If needed, advice should be sought from the specialist centre.

Blood glucose monitoring

The ongoing monitoring of children with CHI in hospital primarily involves regular point of care (fingerprick age >1yr or heelprick age <1yr) glucose testing. The number of tests per day will depend on individual circumstances including response to medications, frequency of feeds and severity of illness. For patients with unstable glucose levels on a high intensity ward such as neonatal

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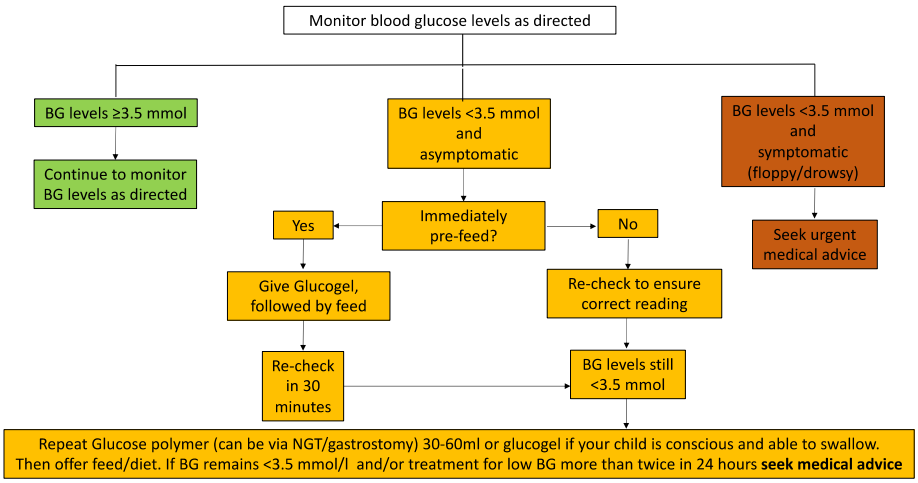

FIGURE 3
Flowchart for blood glucose monitoring for families. BG, blood glucose.

intensive care unit, blood glucose is typically checked 1-2 hourly until stability is achieved. Following stabilisation, most patients require 2-4 hourly blood glucose testing prior to feeds.

Point of care testing is accurate and laboratory glucose testing will not be routinely required. At discharge, patients should receive training in the use of a standard hand held blood glucose meter. Such meters are less accurate than point of care testing devices but are nonetheless widely used as home monitoring devices.

Blood glucose monitoring frequency at home is guided by the severity of illness, propensity to hypoglycaemia and need for adjustment to medications. For instance, when diazoxide dosage is actively reduced, a period of frequent monitoring may be required to reiterate glycaemic stability. There is no universal agreement on what constitutes glycaemic stability; however, by consensus, hypoglycaemic episodes (glucose < 3.5 mmol/L) exceeding two per week would imply imperfect control. For any hypolycaemia at home, parents are advised to follow local hypoglycaemia algorithms. An example of such an algorithm is provided in Figure 3.

Continuous glucose monitoring

CGM in the hospital setting

For most patients, intermittent frequent blood glucose testing provides adequate information for monitoring in hospital. Continuous glucose monitoring (CGM) may be additionally helpful, owing to high frequency sampling providing an almost continuous profile. However, unlike diabetes, evidence for use in CHI is scant (60–62). Accuracy is lower than that of glucometer testing and any hypoglycaemia detected by a CGM device must be verified with a point of care glucometer before treatment is administered (62, 63). In hospital, CGM can be used to provide reassurance about normoglycaemia and thus reduce the number of point of care glucose tests (64). CGM offers additional utility in guiding point of care tests to times of possible hypoglycaemia in between routine tests, a feature that is beneficial in unstable patients.

CGM in the outpatient setting

CGM is not routinely used for home monitoring of CHI and is not supported by nationally agreed funding schemes (outwith individual funding requests). Thus, provision of CGM to patients in the outpatient setting is ascertained on a case by case basis using unpredictable streams of funding (65, 66).

The advantages and disadvantages of CGM in the hospital setting apply equally to the outpatient setting. However, the problems with inaccuracies of current CGM devices are amplified in the outpatient setting detracting from widespread use (66). There is, as yet, no published evidence of the utility of CGM as a standalone tool in reducing hypoglycaemia in CHI (67). CGM at home should be reserved for pattern recognition rather than for the acute detection of hypoglycaemic episodes. Parents should be counselled that CGM does not replace home blood glucose testing and that missed hypoglycamia remains a possibility due to inaccuracy in the present generation of CGM sensors.

Investigations in CHI; role of genetics

Aetiology

Genetic causes for CHI can be found in 45-80% of cases, with the pick-up rate influenced by the screening methods employed by the genetics laboratory (68). The majority of pathogenic variants (69, 70) affect the ATP-sensitive K^+ (KATP) channels in the pancreatic beta-cells, whilst others affect the intracellular proteins and enzymes involved in regulation of the insulin secretion pathway. Patients with a known genetic cause have variable disease trajectories with some achieving remission; however, recessively-acting variants in the KATP channel genes are more likely to be persistent (9) although with the potential for reduced disease severity with time (71). Similarly, those who remain without a genetic diagnosis following testing are more likely to achieve

disease resolution with time, although disease trajectory is inconsistent and duration is unpredictable (72, 73).

CHI can be associated with other metabolic and genetic syndromic conditions such as Turner syndrome, congenital disorders of glycosylation and Beckwith-Wiedemann Syndrome (9, 11, 74, 75). Healthcare professionals managing patients with CHI are therefore advised to consider syndromic diagnoses and seek a genetic review if suspicion is aroused.

Disease-causing variants in the *ABCC8* and *KCJN11* genes which encode the KATP channel, can be inherited by both autosomal dominant and recessive routes. Dominantly inherited variants typically cause diffuse CHI, while recessively inherited variants cause both diffuse and focal CHI. The latter occur with paternal inheritance of a recessively acting KATP channel variant and concomitant loss of the maternal chromosomal 11p15 region within the pancreatic tissue. As a consequence, a focal lesion develops through clonal expansion of mutated β-cells which are dysregulated and secrete excess insulin. By contrast, recessively inherited biallelic variants invariably cause diffuse CHI with typical pancreatic histopathology (76).

Although an exhaustive list of the genetic causes of congenital hyperinsulinism has not been provided in this consensus, a few relatively common causes have been noted. Pathogenic variants in the gene encoding glutamate dehydrogenase (*GLUD1*), are associated with hyperinsulinaemia/hyperammonaemia syndrome where patients develop relatively mild hyperammonaemia (2-3 times the upper limit for serum ammonia) and a post-prandial hypoglycaemia aggravated by protein intake (11, 77, 78). *GLUD1* variants are dominantly acting but are most commonly sporadic in origin. These patients may have seizures which are unrelated to the severity of the hypoglycaemia and may require a protein restricted diet to manage hypoglycaemia (74, 77–79).

Another dominantly inherited cause of hyperinsulinism is due to activating variants in the gene encoding glucokinase (*GCK*). Patients with activating variants in *GCK* may present well beyond infancy and have variable expression in the same family. Recently, variants in a non-coding region of hexokinase 1 (*HK1*) that disrupts a regulatory element controlling gene expression have been described in patients presenting with CHI. Patients show a variable phenotype ranging between transient CHI and severe CHI requiring subtotal pancreatectomy (80).

Genetic investigations should be widened to include variants associated with both CHI and other conditions such as hypopituitarism, as in those with *FOXA2* variants (81). Such genetic testing is best undertaken by targeted gene panel testing where rapid turnaround times are not essential.

The genetic diagnosis of CHI should be confirmed by specialist genomic laboratories with appropriate technological expertise and experience. Accurate genotyping is important for clinical decision making (Figure 2) for optimal outcomes of CHI and for the prevention of unnecessary and harmful investigations that are not required in all cases. For a wider discussion of genetic aetiology and an up to date discussion of laboratory testing practices to investigate underlying cause, comprehensive open access reviews may need to be consulted (68).

Genetic testing strategies

Genetic testing in CHI patients should be undertaken at an early phase of clinical management and guided by the response to first line treatment with diazoxide (9, 11). Rapid *ABCC8/KCNJ11* sequencing is required in severe cases with high GIR and medical instability. Genetic testing should also be considered in those with persistent CHI (> 6 months by consensus) requiring diazoxide > 7mg/kg/day as they may have pathogenic variants in non-KATP channel genes (9). In such patients, gene panel testing should be considered as a less expensive alternative with greater coverage of CHI associated genes, although with a longer reporting turnaround time (68). In those with persistent CHI but diazoxide < 7 mg/kg/day, genetic testing may also be considered if there is no evidence for gradual dose reduction over time. In patients with CHI due to perinatal stress induced hyperinsuinism, genetic testing is not routinely recommended as CHI is unlikely to be of genetic aetiology and very likely to remit (82). It is however important to consider genetic testing, where there is a family history of early diabetes, even if diazoxide responsive, as this may be due to *HNF1A*, *HNF4A* variants (83).

Imaging

18-fluoro-dopa PET imaging with CT or MR modalities is indicated for the detection and localisation of focal lesions (84). As described above, a focal lesion is likely with the detection of a previously reported paternally inherited recessive variant in *ABCC8/KCNJ11*. In such cases, the CHI centre should be contacted for a discussion regarding the requirement, urgency and process for organising a scan. In case of novel variants in *ABCC8/KCNJ11* with no prior information regarding recessive/dominant inheritance, an 18-fluoro-dopa PET-CT/MR scan is also indicated. In this scenario (and in those without gene variants) the need for this scan should be reviewed in the context of illness severity. Patients with stable glycaemic profiles on low to modest doses of diazoxide (i.e. < 10 mg/kg/day) are unlikely to have focal CHI due to KATP channel disruption; in such cases, the decision for 18-fluoro-dopa PET-CT/MR scanning should be a lower priority, unless progressively increasing doses are required to maintain glycaemic stability. It is now recognised that variants in KATP channel genes may be limited to the pancreas and not be present in blood lymphocytes (85). In the absence of a known genetic aetiology from peripheral blood sampling, the presence of persistent hypoglycaemia due to CHI and ongoing need for therapy beyond age 2 years (by consensus) merits the consideration for an 18-fluoro-dopa PET-CT/MR scan (Figure 2).

It is recognised that PET imaging is the best available investigation to localise a focal lesion. However, scan findings are not always concordant with histological outcomes; therefore, treatment teams should be alert to the possibility of potential mislocalisation or a missed lesion on imaging. If a scan outcome is uncertain there is no evidence to suggest a repeat scan, unless the initial scan was of uncertain quality. Alternative radiotracers such as

gallium-exendin have been developed (86), but it is unclear if efficacy overrides that of standard 18-fluoro-dopa PET imaging which has been shown to be highly sensitive (>95%) in the detection of focal lesions (87).

Surgery

In patients with a likely diagnosis of focal CHI (following diagnostic genetics and 18 fluoro-dopa PET-CT/MR scanning (88)), focal lesionectomy at a CHI specialist centre is the treatment of choice. Laparascopic lesionectomy is recommended for removal of lesions in the body or tail of the pancreas, as recovery times are short. However, the choice for minimally invasive laparacopic versus open laparatomy is predicated on individual circumstances and preference of the surgeon. In contrast to focal CHI, subtotal pancreatectomy is considered as a last resort in diffuse CHI not responsive to medical therapy. In all cases, the surgical team should be assisted by the CHI medical team to manage intraoperative and post-operative hypoglycaemia, hyperglycaemia and associated medical conditions. Additionally, frozen section histopathology is advised for all surgical resections, most importantly in focal lesionectomy, to identify and resect the focal lesions (89).

Discharge from hospital

Prior to discharge the infant should undergo a safety fast to ensure a reasonable fasting interval at home. The duration of this fast is intended to replicate a reasonable overnight food free interval that fits into parental routines of care. An age-appropriate safety fast should be performed in all patients with a diagnosis of CHI, including those infants managed with additional CHO alone and those with transient CHI. The safety fast should be considered when the infant feeds at 3 hourly intervals and ideally at 4 hourly intervals with stable glucose levels. The duration of the fast can vary between 6 and 8 hours, depending on the age of the child and expected food free interval, although longer fasts may be preferred by other centres to review capacity for ketogenesis. In contrast to prolonged fasts (favoured by centres outside the UK) that deviate from physiological fasting intervals, a shorter fasting period has greater practical application and is accepted by consensus to convey safety reassurance to families.

During the safety fast, blood glucose should be checked pre feed and then hourly from the 4th hour after the last feed. Absence of end of fast hypoglycaemia (glucose < 3.5mmol/L) is considered a satisfactory outcome. If at any time during the fast, blood glucose dips below 3.5mmol/L, the fast should be discontinued and the patient fed immediately. At this stage the treatment team should review medication and feeds and consider a repeat fast after suitable adjustments. At discharge, an individualised care plan based on local need and family preferences should be discussed and a copy given to the parents. An example of such a plan is provided in Appendix 4.

The role of the extended multidisciplinary team

As CHI is a complex and challenging disorder, an MDT approach is strongly recommended. The MDT composition will depend on local availability of resources but should include paediatric endocrinologists (or specialists in inherited errors of metabolism), specialist nurses and dietitians. The MDT may also include, variably, clinical psychologists, SLT, surgeons, radiologists, nuclear medicine physicians and histopathologists. Specialist nurses have an important role in aiding the understanding for the family, providing them with emotional support during the hospital stay and arranging follow-up at discharge. They are a valuable resource for ward staff managing patients with CHI, providing training, education and expertise in optimising treatment outcomes. The specialist CHI nurse may also assist by providing nurse led outpatient follow up appointments, often through virtual consultations, thereby providing much needed support for parents and families.

The role of the MDT is relevant in long term follow up when neurodevelopmental outcomes and progress through school become more apparent. At all stages, MDT support is helpful for active guidance of treatment and ancillary issues not only for the parents, but also for other professionals around the child, for instance in school. An example of a school plan is provided in Appendix 5.

Long term Management

CHI patients should be monitored in the outpatient clinic 3-6 monthly, to assess growth, development, and responsiveness to treatment. More frequent review, often in virtual consultations, may be required to optimise therapy. If treatment is minimal, e.g. requirement for diazoxide <2-3mg/kg/day or octreotide <3mcg/kg/day with satisfactory glucose profiles, a trial of treatment withdrawal to assess disease remission should be considered. Consensus criteria for ascertaining remission include tolerance of an age-appropriate safety fast with suppressed insulin and robust ketone production (typically >1.5 mmol/L) by laboratory analysis at the end of the fast (71). A satisfactory profile on frequent home glucose monitoring may also suffice in patients with lesser duration and severity of illness, precluding the necessity for hospital admission and a rigorous fast.

Spontaneous resolution is expected in transient CHI related to perinatal stress. The probability of resolution is positively correlated with the absence of gene variants and a satisfactory response to low dose diazoxide. CHI due to pathogenic variants in *HNF4A* is often transient and some cases with dominantly acting *ABCC8/KCNJ11* may also resolve, but do so variably (90–92). Some infants with *HNF4A*, *HNF1A* and dominantly acting *ABCC8* variants may develop diabetes later in life, and therefore require advice and monitoring (83, 93) Some children develop idiopathic ketotic hypoglycaemia following resolution of CHI (94, 95) while in others, hypoglycaemia may recur. Therefore, except for perinatal stress related CHI, a follow up plan, even if infrequent, is recommended. Parents are advised to monitor glucose at home

during intercurrent illness episodes, particularly if reluctant to have food or drink by mouth.

Neurodevelopmental outcomes

Abnormal neurodevelopmental outcomes are present in a significant number of children with both persistent and transient forms of CHI (96, 97) with frequency up to 48% in those with severe forms. While it is important to prevent neuroglycopaenia, it is also important to assess developmental progress in follow up. A developmental follow up schedule alongside medical review will enable screening for early problems, thereby facilitating early referral to community services for rapid intervention. Screening for neurodevelopmental outcomes could be undertaken from age one year (Appendix 6), although evolving abnormalities may present later and ongoing neurodevelopmental assessment should be considered, especially with parental concerns (98). In older children, and in those with screening deficits, standardised assessment of cognitive function may be required. Brain imaging is not usually part of standard clinical review as anatomical abnormalities do not necessarily correlate with functional outcomes. However, in those with severe neuroglycopaenia, visual cortical function loss or recurrent seizures, brain imaging may be helpful for review by paediatric neurologists.

Post pancreatectomy diabetes

Following sub-total pancreactomy, diabetes is common, with some cases developing diabetes shortly after surgery and some many years later. In the majority, diabetes develops within 10 years after surgery (73, 99, 100). Monitoring of pancreatic exocrine function is also required following sub-total pancreactomy. Faecal elastase should be measured annually. Pancreatic enzyme replacement therapy such as Creon®, should be considered, although not all patients will be symptomatic despite a low faecal elastase level (100).

Future therapies

Standard therapies are currently limited to a choice of diazoxide and octreotide and are often complicated by side effects (9). This has prompted the use of alternative therapies in observational studies such as sirolimus (101). However, sirolimus can be associated with side effects and should be used with caution in exceptional circumstances (102–104). The use of nifedipine, a calcium channel blocker, is not generally advocated for use (105).

New therapies are currently in clinical trials; these include soluble glucagon analogues (26, 106), long-acting glucagon, somatostatin receptor type 5 (SSTR5) agonist, monoclonal allosteric antibodies targeting the insulin receptor (107) and glucagon-like peptide-1 receptor (GLP-1r) antagonists. However, these are not yet available for routine clinical use.

Parent perspectives

The Children's Hyperinsulinism Charity (CHC) is the registered patient charity for CHI in the UK. They offer support for parents and families living with CHI and interact and collaborate with clinical teams and organisations involved in healthcare provision for CHI patients. Useful information and resources can also be found on their website. (www.hyperinsulinism.co.uk), which is very helpful for parents especially during the initial diagnosis. As an important patient voice, they represent the perceptions of the community, represented as quotes ad verbatim in Figure 4. These quotes represent feelings resulting from a combination of stress and anxiety at diagnosis, prolonged hospital stay, inadequate bonding, sleep deprivation, fear of alarms, information gap, preparing medications, feeding problems, monitoring requirements, uncertain

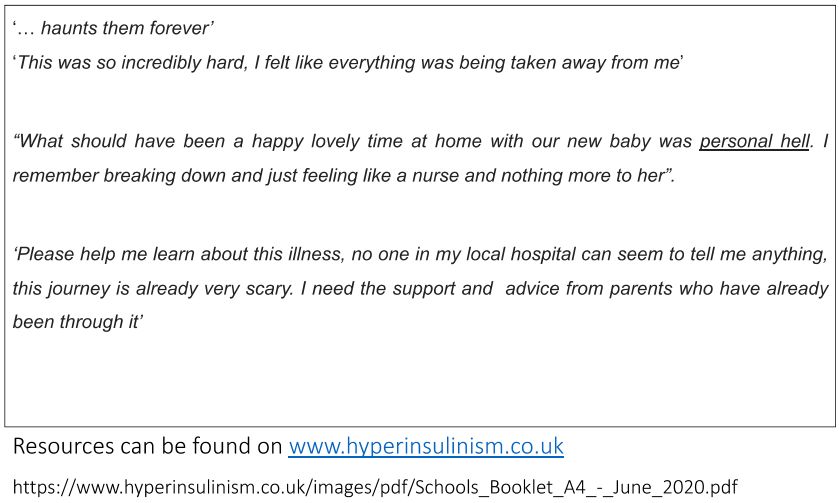

FIGURE 4
Quotes from parents describing their feelings in living with children with CHI.

prognosis and many other factors. Parents often have to come to terms with a potential long-term illness and disability with strenuous feeding and sleep schedules in their child, They also have to live with with long-term caring responsibilities and realise that the burden of care for a child with CHI is much greater than another child of the same age, thereby adversely affecting the quality of family life. Treatment teams should recognise the strength of such feelings and work closely with organisations like the CHC as an important component in the networked care of CHI.

Conclusions

CHI is a complex and difficult disease that usually presents in infancy but has long term effects resulting in multiple problems manifesting throughout childhood and later life. A UK wide consensus of healthcare professionals and patient representatives involved and experienced in the care of CHI patients has synthesised current evidence, practical considerations and harmonised practices to provide narratives and recommendations outlining a standardised approach to patient care with emphasis on partnership and collaboration involving treatment teams, parents, young people and relevant organisations.

Author contributions

MGS devised this project and led on writing and coordination. RC and PC contributed to drafting and review of section on surgery. All authors contributed to the article and approved the submitted version.

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Conflict of interest

IB has received honoraria for advisory opinion from Merck and Diurnal Pharmaceuticals. He is the UK CI for clinical trials funded by Zealand Pharmaceuticals and has received grant funding from Merck, Diurnal and Crinetics Pharmaceuticals. IB is the Chair of the BSPED-NIHR Clinical Studies Group and the Chair of the ESPE Communications Committee. He is a co-opted member of the NICE Head Injury Update Committee. IB has advisory roles with several patient organisations including the Pituitary Foundation, Living with CAH, CHC and CHI. MGS has received funding/speaker honorium from Novo Nordisk, Sandoz, Pfizer and received honorarium for consultancies/advisory boards from Novo Nordisk, Pfizer and Merck. RK has received grant funding and honoraria for advisory opinion from Merck.

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.2023.1231043/full#supplementary-material

References

1. 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):1542–51. doi: 10.1210/clinem/dgab024

2. Galcheva S, Demirbilek H, Al-Khawaga S, Hussain K. The genetic and molecular mechanisms of congenital hyperinsulinism. *Front Endocrinol* (2019) 10. doi: 10.3389/fendo.2019.00111

3. Otonkoski T, Ammälä 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

4. Glaser B, Thornton P, Otonkoski T, Junien C. Genetics of neonatal hyperinsulinism. *Arch Dis Child Fetal Neonatal Ed* (2000) 82(2):F79–86. doi: 10.1136/fn.82.2.F79

5. Otonkoski T, Näntö-Salonen K, Seppänen M, Veijola R, Huopio H, Hussain K, et al. Noninvasive diagnosis of focal hyperinsulinism of infancy with [18F]-DOPA positron emission tomography. *Diabetes* (2006) 55(1):13–8. doi: 10.2337/diabetes.55.01.06.db05-1128

6. 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

7. 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

8. Eljamel S, Griffiths A, Evans J, Banerjee I, Hussain K, Thompson R. The burden of congenital hyperinsulinism in the United Kingdom: a cost of illness study. *Orphanet J Rare Dis* (2018) 13(1):123. doi: 10.1186/s13023-018-0867-6

9. 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(1):9–21. doi: 10.1111/dme.13823

10. De Cosio AP, Thornton P. Current and emerging agents for the treatment of hypoglycemia in patients with congenital hyperinsulinism. *Paediatr Drugs* (2019) 21 (3):123–36. doi: 10.1007/s40272-019-00334-w

11. Giri D, Hawton K, Senniappan S. Congenital hyperinsulinism: recent updates on molecular mechanisms, diagnosis and management. *J Pediatr Endocrinol Metab* (2021) 35(3):279–96. doi: 10.1515/jpem-2021-0369

12. World Health Organisation. *Mean fasting blood glucose (mmol/L)(age-standardized estimate)*. (2023).

13. van Kempen A, Eskes PF, Nuytemans D, van der Lee JH, Dijksman LM, van Veenendaal NR, et al. Lower versus traditional treatment threshold for neonatal hypoglycemia. *N Engl J Med* (2020) 382(6):534–44. doi: 10.1056/NEJMoa1905593

14. McKinlay CJD, Alsweiler JM, Anstice NS, Burakevych N, Chakraborty A, Chase JG, et al. Association of neonatal glycemia with neurodevelopmental outcomes at 4.5 years. *JAMA Pediatr* (2017) 171(10):972–83. doi: 10.1001/jamapediatrics.2017.1579

15. Shah R, Dai DWT, Alsweiler JM, Brown GTL, Chase JG, Gamble GD, et al. Association of neonatal hypoglycemia with academic performance in mid-childhood. *JAMA* (2022) 327(12):1158–70. doi: 10.1001/jama.2022.0992

16. 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

17. 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

18. (BAPM). *Identification and Management of Neonatal Hypoglycaemia in the Full Term Infant*. (2017).

19. de Lonlay P, Giurgea I, Touati G, Saudubray JM. Neonatal hypoglycaemia: aetiologies. *Semin Neonatol* (2004) 9(1):49–58. doi: 10.1016/j.siny.2003.08.002

20. NZ. *Online Glucose Requirement Calculator*. Available at: https://starship.org.nz/health-professionals/calculators/glucose-calculator/.

21. Solimano A. Drop that calculator! You can easily calculate the glucose infusion rate in your head, and should! *Paediatr Child Health* (2020) 25(4):199–200. doi: 10.1093/pch/pxz115

22. Siersbæk J, Larsen AR, Nybo M, Christesen HT. A sensitive plasma insulin immunoassay to establish the diagnosis of congenital hyperinsulinism. *Front Endocrinol (Lausanne)* (2020) 11:614993. doi: 10.3389/fendo.2020.614993

23. Ferrara C, Patel P, Becker S, Stanley CA, Kelly A. Biomarkers of Insulin for the Diagnosis of Hyperinsulinemic Hypoglycemia in Infants and Children. (2016) 268, 212–219. doi: 10.1016/j.jpeds.2015.09.045

24. Fawke J, Wyllie J, Madar J, Ainsworth S, Tinnion R, Chittick R, et al. *Newborn resuscitation and support of transition of infants at birth Guidelines* (2021). Available at: https://www.resus.org.uk/library/2021-resuscitation-guidelines/newborn-resuscitation-and-support-transition-infants-birth#drugs.

25. Salomon-Estebanez M, Yau D, Dunne MJ, Worth C, Birch S, Walewski JL, et al. Efficacy of dose-titrated glucagon infusions in the management of congenital hyperinsulinism: A case series. *Front Endocrinol (Lausanne)* (2020) 11:441. doi: 10.3389/fendo.2020.00441

26. Vibora PI, Arroyo-Díez F. New uses and formulations of glucagon for hypoglycaemia. *Drugs Context* (2019) 8:1–10. doi: 10.7573/dic.212599

27. Hawkes CP, Lado JJ, Givler S, De Leon DD. The effect of continuous intravenous glucagon on glucose requirements in infants with congenital hyperinsulinism. *JIMD Rep* (2019) 45:45–50. doi: 10.1007/8904_2018_140

28. Mohnike K, Blankenstein O, Pfuetzner A, Pötzsch S, Schober E, Steiner S, et al. Long-term non-surgical therapy of severe persistent congenital hyperinsulinism with glucagon. *Horm Res* (2008) 70(1):59–64. doi: 10.1159/000129680

29. Chen SC, Dastamani A, Pintus D, Yau D, Aftab S, Bath L, et al. Diazoxide-induced pulmonary hypertension in hyperinsulinaemic hypoglycaemia: Recommendations from a multicentre study in the United Kingdom. *Clin Endocrinol (Oxf)* (2019) 91(6):770–5. doi: 10.1111/cen.14096

30. Yau D, Salomon-Estebanez M, Chinoy A, Grainger J, Craigie RJ, Padidela R, et al. Central venous catheter-associated thrombosis in children with congenital hyperinsulinism. *Endocrinol Diabetes Metab Case Rep* (2019) 2019(1). doi: 10.1530/EDM-19-0032

31. 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

32. 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

33. Taylor-Miller T, Houghton J, Munyard P, Kumar Y, Puvirajasinghe C, Giri D. Congenital hyperinsulinism due to compound heterozygous mutations in ABCC8 responsive to diazoxide therapy. *J Pediatr Endocrinol Metab* (2020) 33(5):671–4. doi: 10.1515/jpem-2019-0457

34. 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

35. Herrera A, Vajravelu ME, Givler S, Mitteer L, Avitabile CM, Lord K, et al. Prevalence of adverse events in children with congenital hyperinsulinism treated with diazoxide. *J Clin Endocrinol Metab* (2018) 103(12):4365–72. doi: 10.1210/jc.2018-01613

36. Chen X, Feng L, Yao H, Yang L, Qin Y. Efficacy and safety of diazoxide for treating hyperinsulinemic hypoglycemia: A systematic review and meta-analysis. *PLoS One* (2021) 16(2):e0246463. doi: 10.1371/journal.pone.0246463

37. Chandran S, Mei Chien C, Saffari SE, Rajadurai VS, Yap F. Safety and efficacy of low-dose diazoxide in small-for-gestational-age infants with hyperinsulinaemic hypoglycaemia. *Arch Dis Childhood - Fetal Neonatal Edition* (2022) 107(4):359–63. doi: 10.1136/archdischild-2021-322845

38. Neha Malhotra BG, Athanasakopoulou S, Gilbert C, Morgan K, Dattani M, Dastamani A. Optimizing Diazoxide dose for the treatment of newborns with hyperinsulinemic hypoglycaemia (HH)-60th Annual Meeting of the European Society for Paediatric Endocrinology (ESPE). *Hormone Res Paediatrics* (2022) 95 (suppl 2):1–616.

39. Keyes ML, Healy H, Sparger KA, Orth LE, Geha M, Roumiantsev S, et al. "Necrotizing enterocolitis in neonates with hyperinsulinemic hypoglycemia treated with diazoxide. *Pediatrics* (2021) 147(2). doi: 10.1542/peds.2019-3202

40. Prado LA, Castro M, Weisz DE, Jain A, Belik J. Necrotising enterocolitis in newborns receiving diazoxide. *Arch Dis Child Fetal Neonatal Ed* (2021) 106(3):306–10. doi: 10.1136/archdischild-2020-319057

41. Avatapalle B, Banerjee I, Malaiya N, Padidela R. Echocardiography monitoring for diazoxide induced pericardial effusion. *BMJ Case Rep* (2012) 2012. doi: 10.1136/bcr.03.2012.6110

42. Ponmani C, Gannon H, Hussain K, Senniappan S. Paradoxical hypoglycaemia associated with diazoxide therapy for hyperinsulinaemic hypoglycaemia. *Horm Res Paediatr* (2013) 80(2):129–33. doi: 10.1159/000353773

43. van Albada ME, Mohnike K, Dunne MJ, Banerjee I, Betz SF. Somatostatin receptors in congenital hyperinsulinism: Biology to bedside. *Front Endocrinol (Lausanne)* (2022) 13:921357. doi: 10.3389/fendo.2022.921357

44. Hosokawa Y, Kawakita R, Yokoya S, Ogata T, Ozono K, Arisaka O, et al. Efficacy and safety of octreotide for the treatment of congenital hyperinsulinism: a prospective, open-label clinical trial and an observational study in Japan using a nationwide registry. *Endocrine J* (2017) 64(9):867–80. doi: 10.1507/endocrj.EJ17-0024

45. Testoni D, Hornik CP, Neely ML, Yang Q, McMahon AW, Clark RH, et al. Safety of octreotide in hospitalized infants. *Early Hum Dev* (2015) 91(7):387–92. doi: 10.1016/j.earlhumdev.2015.04.008

46. McMahon AW, Wharton GT, Thornton P, De Leon DD. Octreotide use and safety in infants with hyperinsulinism. *Pharmacoepidemiol Drug Saf* (2017) 26(1):26–31. doi: 10.1002/pds.4144

47. Chandran S, Agarwal A, Llanora GV, Chua MC. Necrotising enterocolitis in a newborn infant treated with octreotide for chylous effusion: is octreotide safe? *BMJ Case Rep* (2020) 13(2). doi: 10.1136/bcr-2019-232062

48. Demirbilek H, Shah P, Arya VB, Hinchey L, Flanagan SE, Ellard S, et al. Long-term follow-up of children with congenital hyperinsulinism on octreotide therapy. *J Clin Endocrinol Metab* (2014) 99(10):3660–7. doi: 10.1210/jc.2014-1866

49. 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(5):355–60. doi: 10.1159/000439131

50. Dastamani A, Güemes M, Pitfield C, Morgan K, Rajab M, Rottenburger C, et al. The use of a long-acting somatostatin analogue (Lanreotide) in three children with focal forms of congenital hyperinsulinaemic hypoglycaemia. *Hormone Res paediatrics* (2019) 91(1):56–61. doi: 10.1159/000491101

51. Haris B, Saraswathi S, Hussain K. Somatostatin analogues for the treatment of hyperinsulinaemic hypoglycaemia. *Ther Adv Endocrinol Metab* (2020) 11:2042018820965068. doi: 10.1177/2042018820965068

52. Cuff H, Lord K, Ballester L, Scully T, Stewart N, De Leon DD. The use of lanreotide in the treatment of congenital hyperinsulinism. *J Clin Endocrinol Metab* (2022) 107(8):e3115–20. doi: 10.1210/clinem/dgac322

53. 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(4):278–83. doi: 10.6065/apem.2142010.005

54. Shaw V. *Clinical Paediatric Dietetics*. Wiley-Blackwell (2014).

55. Della Pepa G, Vetrani C, Lupoli R, Massimino E, Lembo E, Riccardi G, et al. Uncooked cornstarch for the prevention of hypoglycemic events. *Crit Rev Food Sci Nutr* (2022) 62(12):3250–63. doi: 10.1080/10408398.2020.1864617

56. Evans S, Shelton F, Holden C, Daly A, Hopkins V, MacDonald A. Monitoring of home safety issues in children on enteral feeds with inherited metabolic disorders. *Arch Dis Child* (2010) 95(9):668–72. doi: 10.1136/adc.2008.148338

57. 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. doi: 10.3389/fendo.2016.00008

58. Worth C, Hall C, Wilson S, Gilligan N, O'Shea E, Salomon-Estebanez M, et al. "Delayed resolution of feeding problems in patients with congenital hyperinsulinism. *Front Endocrinol* (2020) 11. doi: 10.3389/fendo.2020.00143

59. Hall C, Banerjee I. *Feeding Problems in Congenital Hyperinsulinism. Congenital Hyperinsulinism: A Practical Guide to Diagnosis and Management*. De León-Crutchlow DD, Stanley CA, editors. Cham: Springer International Publishing (2019) p. 143–54.

60. Worth C, Dunne M, Ghosh A, Harper S, Banerjee I. Continuous glucose monitoring for hypoglycaemia in children: Perspectives in 2020. *Pediatr Diabetes* (2020) 21(5):697–706. doi: 10.1111/pedi.13029

61. Tropeano Y, Preetha P, Clare G, Morgan K, Doodson L, Dastamani A. Efficacy of use of continuous glucose monitoring system in patients with congenital hyperinsulinism. *Hormone Res Paediatrics* (2022) 82 Supplement1.

62. Worth C, Hoskyns L, Salomon-Estebanez M, Nutter PW, Harper S, Derks TGJ, et al. Continuous glucose monitoring for children with hypoglycaemia: Evidence in 2023. *Front Endocrinol (Lausanne)* (2023) 14:1116864. doi: 10.3389/fendo.2023.1116864

63. 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(1) 3.

64. Rayannavar A, Elci OU, Mitteer L, De León DD. Continuous glucose monitoring systems: are they useful for evaluating glycemic control in children with hyperinsulinism? *Hormone Res Paediatrics* (2019) 92(5):319–27. doi: 10.1159/000506230

65. Ng SM, Dearman S, Fisher M, Mushtaq T, Randell T. Case for funding of continuous glucose monitoring systems for patients with recurrent hypoglycaemia. *Arch Dis Child* (2022). doi: 10.1136/archdischild-2022-323872

66. Raskin J, Pasquini TLS, Bose S, Tallis D, Schmitt J. Congenital hyperinsulinism international: A community focused on improving the lives of people living with congenital hyperinsulinism. *Front Endocrinol* (2022) 13. doi: 10.3389/fendo.2022.886552

67. Worth C, Dunne MJ, Salomon-Estebanez M, Harper S, Nutter PW, Dastamani A, et al. The hypoglycaemia error grid: A UK-wide consensus on CGM accuracy assessment in hyperinsulinism. *Front Endocrinol (Lausanne)* (2022) 13:1016072. doi: 10.3389/fendo.2022.1016072

68. 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

69. 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

70. 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):E355–363. doi: 10.1210/jc.2012-2169

71. Salomon-Estebanez M, Flanagan SE, Ellard S, Rigby L, Bowden L, Mohamed Z, et al. Conservatively treated Congenital Hyperinsulinism (CHI) due to K-ATP channel gene mutations: reducing severity over time. *Orphanet J rare Dis* (2016) 11(1):1–11. doi: 10.1186/s13023-016-0547-3

72. Banerjee I, Skae M, Flanagan S, 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. doi: 10.1530/EJE-10-1136

73. El Tonbary K, Robinson P, Banerjee I, Shaikh MG. Congenital hyperinsulinism: management and outcome, a single tertiary centre experience. *Eur J Pediatr* (2020) 179:947–52. doi: 10.1007/s00431-020-03581-z

74. Demirbilek H, Hussain K. Congenital hyperinsulinism: diagnosis and treatment update. *J Clin Res Pediatr Endocrinol* (2017) 9(Suppl 2):69.

75. 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

76. Han B, Newbould M, Batra G, Cheesman E, Craigie RJ, Mohamed Z, et al. Enhanced islet cell nucleomegaly defines diffuse congenital hyperinsulinism in infancy but not other forms of the disease. *Am J Clin Pathol* (2016) 145(6):757–68. doi: 10.1093/ajcp/aqw075

77. 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

78. Aftab S, Gubaeva D, Houghton JAL, Dastamani A, Sotiridou E, Gilbert C, et al. Spectrum of neuro-developmental disorders in children with congenital hyperinsulinism due to activating mutations in GLUD1. *Endocr Connect* (2022). doi: 10.1530/EC-22-0008

79. Hsu BY, Kelly A, Thornton PS, Greenberg CR, Dilling LA, Stanley CA. Protein-sensitive and fasting hypoglycemia in children with the hyperinsulinism/hyperammonemia syndrome. *J Pediatr* (2001) 138(3):383–9. doi: 10.1067/mpd.2001.111818

80. Wakeling MN, Owens NDL, Hopkinson JR, Johnson MB, Houghton JAL, Dastamani A, et al. Non-coding variants disrupting a tissue-specific regulatory element in HK1 cause congenital hyperinsulinism. *Nat Genet* (2022) 54(11):1615–20. doi: 10.1038/s41588-022-01204-x

81. 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

82. Arya VB, Flanagan SE, Kumaran A, Shield JP, Ellard S, Hussain K, et al. Clinical and molecular characterisation of hyperinsulinaemic hypoglycaemia in infants born small-for-gestational age. *Arch Dis Child Fetal Neonatal Ed* (2013) 98(4):F356–358. doi: 10.1136/archdischild-2012-302880

83. 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

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

85. 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

86. Boss M, Rottenburger C, Brenner W, Blankenstein O, Prasad V, Prasad S, et al. (68)Ga-NODAGA-exendin-4 PET improves the detection of focal congenital hyperinsulinism. *J Nucl Med* (2021). doi: 10.2967/jnumed.121.262327

87. Sathya Murthi B, Yau D, Alam S, Salomon Estebanez M, Banerjee I, Ross C, et al. Role of [18F] DOPA PET-CT in suspected congenital Hyperinsulinism. *J Nucl Med* (2022) 63(supplement 2):2392–2.

88. Dastamani A, Yau D, Gilbert C, Morgan K, De Coppi P, Craigie RJ, et al. Variation in glycemic outcomes in focal forms of congenital hyperinsulinism—The UK perspective. *J Endocrine Soc* (2022) 6(6). doi: 10.1210/jendso/bvac033

89. Craigie RJ, Salomon-Estebanez M, Yau D, Han B, Mal W, Newbould M, et al. Clinical diversity in focal congenital hyperinsulinism in infancy correlates with histological heterogeneity of islet cell lesions. *Front Endocrinol (Lausanne)* (2018) 9:619. doi: 10.3389/fendo.2018.00619

90. 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

91. Flanagan S, Kapoor R, Banerjee I, Hall C, Smith V, Hussain K, et al. Dominantly acting ABCC8 mutations in patients with medically unresponsive hyperinsulinaemic hypoglycaemia. *Clin Genet* (2011) 79(6):582–7. doi: 10.1111/j.1399-0004.2010.01476.x

92. Kapoor RR, Flanagan SE, James CT, McKiernan J, Thomas AM, Harmer SC, et al. Hyperinsulinaemic hypoglycaemia and diabetes mellitus due to dominant ABCC8/KCNJ11 mutations. *Diabetologia* (2011) 54(10):2575–83. doi: 10.1007/s00125-011-2207-4

93. Işık E, Demirbilek H, Houghton JA, Ellard S, Flanagan SE, Hussain K. Congenital Hyperinsulinism and Evolution to Sulfonylurearesponsive Diabetes Later in Life due to a Novel Homozygous p.L171F ABCC8 Mutation. *J Clin Res Pediatr Endocrinol* (2019) 11(1):82–7. doi: 10.4274/jcrpe.galenos.2018.2018.0077

94. Kochar IS, Hussain K. From hyperinsulinaemic hypoglycaemia to ketotic hypoglycaemia: the range of glucose abnormalities in patients born with intrauterine growth retardation. *Eur J Pediatr* (2007) 166(10):1003–7. doi: 10.1007/s00431-006-0371-1

95. Patil P, Giri D, Didi M, Senniappan S. Ketotic hypoglycemia in children with previous transient congenital hyperinsulinism. *Indian Pediatr* (2018) 55:167–8.

96. 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

97. 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

98. Salomon-Estebanez M, Mohamed Z, Michaelidou M, Collins H, Rigby L, Skae M, et al. Vineland adaptive behavior scales to identify neurodevelopmental problems in children with Congenital Hyperinsulinism (CHI). *Orphanet J Rare Dis* (2017) 12(1):96. doi: 10.1186/s13023-017-0648-7

99. 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(2):198–203. doi: 10.2337/dc11-1296

100. 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

101. 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):1131–7. doi: 10.1056/NEJMoa1310967

102. 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

103. Banerjee I, De Leon D, Dunne MJ. Extreme caution on the use of sirolimus for the congenital hyperinsulinism in infancy patient. *Orphanet J Rare Dis* (2017) 12(1) 70. doi: 10.1186/s13023-017-0621-5

104. Maria G, Antonia D, Michael A, Kate M, Sian E, Sarah FE, 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

105. Guemes 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

106. Shin W, K. J, Lee G, Arbelaez MA, Banerjee I, Dastamani A, et al. An ongoing phase 2 study of HM15136, a novel long-acting glucagon analogue, in subjects with congenital hyperinsulinism (ACHIEVE, outline protocol)." 60th annual meeting of the European society for paediatric endocrinology (ESPE). *Horm Res Paediatr* (2022) 95(suppl 2):1–616. doi: 10.1159/000525606

107. Huseyin Demirbilek MM, Galcheva S, Dastamani A, Thornton P, De Leon D, Raskin J, et al. Results from a global, multi-center, phase 2b study (RIZE) in congenital hyperinsulinism: characterization of a high unmet treatment need and glycemic response to RZ358-60th annual meeting of the European society for paediatric endocrinology (ESPE). *Hormone Res Paediatrics* (2022) 95(suppl 2):1–616.

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Genotype-phenotype correlation in Taiwanese children with diazoxide-unresponsive congenital hyperinsulinism

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Objective: Congenital hyperinsulinism (CHI) is a group of clinically and genetically heterogeneous disorders characterized by dysregulated insulin secretion. The aim of the study was to elucidate genetic etiologies of Taiwanese children with the most severe diazoxide-unresponsive CHI and analyze their genotype-phenotype correlations.

Methods: We combined Sanger with whole exome sequencing (WES) to analyze CHI-related genes. The allele frequency of the most common variant was estimated by single-nucleotide polymorphism haplotype analysis. The functional effects of the ATP-sensitive potassium (K_{ATP}) channel variants were assessed using patch clamp recording and Western blot.

Results: Nine of 13 (69%) patients with ten different pathogenic variants (7 in *ABCC8*, 2 in *KCNJ11* and 1 in *GCK*) were identified by the combined sequencing. The variant *ABCC8* p.T1042QfsX75 identified in three probands was located in a specific haplotype. Functional study revealed the human SUR1 (hSUR1)-L366F K_{ATP} channels failed to respond to intracellular MgADP and diazoxide while hSUR1-R797Q and hSUR1-R1393C K_{ATP} channels were defective in trafficking. One patient had a *de novo* dominant mutation in the *GCK* gene (p.I211F), and WES revealed mosaicism of this variant from another patient.

Conclusion: Pathogenic variants in K_{ATP} channels are the most common underlying cause of diazoxide-unresponsive CHI in the Taiwanese cohort. The

p.T1042QfsX75 variant in the *ABCC8* gene is highly suggestive of a founder effect. The I211F mutation in the *GCK* gene and three rare SUR1 variants associated with defective gating (p.L366F) or traffic (p.R797Q and p.R1393C) K_{ATP} channels are also associated with the diazoxide-unresponsive phenotype.

KEYWORDS

congenital hyperinsulinism, *ABCC8*, *KCNJ11*, *GCK*, K_{ATP} channel, founder mutation

1 Introduction

Congenital hyperinsulinism (CHI) is a rare inherited disorder in the metabolism of glucose characterized by dysregulation in insulin secretion. Patients present with severe hypoglycemia associated with inappropriate insulin secretion. Delayed diagnosis may result in irreversible brain damage due to prolonged hypoglycemia (1). CHI has a heterogeneous genetic etiology, and pathogenic variants in *ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC16A1*, *HNF4A*, *HNF1A*, *HK1*, and *INSR* genes have been identified to cause isolated, persistent CHI (2).

In pancreatic β cells, the ATP-sensitive potassium (K_{ATP}) channel plays a critical role in coupling changes in the plasma glucose concentration to electrical excitability and insulin release (3). The K_{ATP} channel is an octameric complex consisting of four subunits of the pore-forming potassium channel subunit (Kir6.2) and four sulfonylurea receptor 1 (SUR1) subunits, which are encoded by *KCNJ11* and *ABCC8* genes, respectively. At the resting metabolic state, opening of the K_{ATP} channels hyperpolarizes the cell membrane. As circulating glucose levels rise, glucose enters the pancreatic β cells and increases the intracellular ATP concentration ([ATP]i) through the glucokinase-mediated glycolysis pathway. This elevated [ATP]i leads to the closure of K_{ATP} channels, causing the depolarization of pancreatic β cells and resulting in insulin secretion. In addition to ATP, K_{ATP} channels are modulated by different intracellular molecules, including the phospholipid PIP_2 and magnesium (Mg)-nucleotides such as MgATP and MgADP (4–6).

CHI is classified as diffuse, focal, or atypical form depending on the pancreatic histological assessment. The diffuse form of CHI is generally associated with recessive loss-of-function variants in the *ABCC8* and *KCNJ11* genes. Patients with this condition are usually unresponsive to potassium channel openers (e.g., diazoxide), and extensive pancreatectomy is required to ameliorate hypoglycemia. Most cases of focal CHI are related to paternally inherited pathogenic variants in either *ABCC8* or *KCNJ11* and somatic loss of the maternal 11p15 region (7, 8). Although the clinical manifestations of patients with focal and diffuse CHI due to pathogenic variants in *ABCC8* or *KCNJ11* are indistinguishable, those with focal CHI can be cured by excision of the lesion (9). Atypical CHI is not easily classified as diffuse or focal form and is characterized by morphological mosaicism with hyperfunctional islets confined to discrete regions of the pancreas (10, 11). Although mosaic interstitial paternal uniparental isodisomy for chromosome 11p15.1 with an *ABCC8* gene mutation has been reported to explain one of the genetic mechanisms (10), the genetic background of most cases with atypical CHI remains unclear (11). Dominant mutations in *ABCC8* and *KCNJ11* genes, as well as disease-causing variants in other CHI-related genes, have been frequently associated with mild and diazoxide-responsive CHI (2).

In this study, we performed molecular analyses to characterize a cohort of thirteen Taiwanese children with diazoxide-unresponsive CHI. Their pathological findings were analyzed to assess genotype-phenotype correlations. Functional status of rare K_{ATP} variants in these patients was studied and the founder effect of the most prevalent pathogenic variant was elucidated.

2 Materials and methods

2.1 Patients

The diagnostic criteria for CHI were modified from those of the European Network for Research into Hyperinsulinism (12, 13): [1] laboratory blood glucose level of <50 mg/dL; [2] glucose requirement >6-8 mg/kg/min to maintain a blood glucose level of >50 mg/dL; [3] detectable insulin at the point of hypoglycemia, with raised C-peptide; [4] inappropriately low blood ketone body concentration at the time of hypoglycemia; and [5] glycemic response after glucagon administration during hypoglycemia. Patients with insulinoma, transient or syndromic hyperinsulinemic hypoglycemia were excluded. Thirteen children diagnosed with diazoxide-unresponsive CHI between 1990 and 2016 at National Taiwan University Hospital were enrolled. Non-responsiveness to diazoxide was defined as failure to keep blood glucose level >50 mg/dL in a five-day trial of oral diazoxide therapy at 15 mg/kg/day (neonates) or 10 mg/kg/day (infants) divided into three doses (14).

2.2 DNA isolation and genotyping

Genomic DNA was extracted using the commercially available QIAamp DNA Blood Mini Kit (QIAGEN, Germany). Direct Sanger sequencing of *ABCC8* and *KCNJ11* was performed. When no pathogenic variants were identified, we sequenced other CHI-

related genes. PCR primers for the coding exons were designed using the Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/). The PCR products were sequenced using a BigDye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and analyzed by the 3130xl genetic analyzer (Applied Biosystems). The sequences were compared with published sequence data (*ABCC8*-NM_000352.3, *KCNJ11*-NM_000525.3, *GLUD1*-NM_005271.3, *GCK*-NM_000162.3, *HADH*-NM_005327.4, *SLC16A1*-NM_003051.3, *HNF4A*-NM_000457.3, *HNF1A*-NM_000545.6). The significance of the identified variants was accessed by Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/). Possible large genomic structural variant in *ABCC8* was analyzed using the SALSA multiplex ligation-dependent probe amplification (MLPA) kit, P117 (MRC Holland, Amsterdam, Netherlands). The pathogenicity of genetic variants was classified using the American College of Medical Genetics and Genomics (ACMG) criteria (15).

Whole exome sequencing (WES) was performed for the cases if no disease-causing variants were identified by Sanger sequencing. The pipeline for variant calling and filtering analysis of WES data was performed as previously described (16). The filtered variants were queried from 35 genes involved in pancreatic islet β cell function and insulin secretion (2, 17) (Supplementary Table 1). The potential pathogenic variants were validated by Sanger sequencing, and segregation analysis was performed.

2.3 Single-nucleotide polymorphisms genotyping

Three probands carrying the c.3124_3126delACCinsCAGC CAGGAACTG variant (rs786204542) in the *ABCC8* gene and a total of 68,978 control samples were genotyped using the TWBv2 SNP array (Thermo Fisher Scientific, Inc., Santa Clara, CA, USA), a genome-wide SNP chip designed in 2017 for the Taiwanese Han population (18). After genotyping, SNPs fulfilling the following criteria were included for haplotype analysis: [1] SNPs located in the surrounding region (± 100 kb) of the *ABCC8* gene; [2] with a minor allele frequency of >0.5% (excluding the rs786204542 variant); [3] a call rate >95%; and [4] satisfying the Hardy–Weinberg equilibrium (HWE) ($P > 0.0001$) (19).

2.4 Haplotype estimation and statistical analysis

Two scales of chromosomal haplotype regions among the cases and controls generated from the genotyped data were tested: [1] the bilateral 50 Kb regions flanking rs786204542, and [2] the bilateral 20 SNPs [110.3 Kb upstream (rs117210015) and 62.8 Kb downstream (rs2237969)] flanking rs786204542. Haplotype frequencies were estimated using the SAS/Genetics HAPLOTYPE PROCEDURE (SAS Institute, Cary, NC, USA) and the stepwise expectation-maximization (EM) algorithm (19, 20). The probability of individual's particular haplotype was calculated given the haplotype frequency reached the maximum likelihood estimations (MLEs) by stepwise EM algorithm. Individual haplotype pairs were included in the analysis when the probability of the event was higher than 0.7 (21). The differences in haplotype frequencies between the case and control groups were assessed using Fisher's exact test. The permutation test was used to estimate the likelihood that the three cases carry the same rare extended haplotype containing rs786204542. To drive the empirical distribution while preserving the structure of the genotype data, we performed 100,000 or 1,000,000 replications by shuffling the case-control status and calculated permutation *P* values by comparing haplotype frequencies between cases and controls (22).

2.5 Electrophysiological and biochemical analysis

Human Kir6.2 (hKir6.2), and SUR1 (hSUR1) (Origene, Rockville, MD, USA) were cloned into the pcDNA3 plasmid as described previously (23, 24). Site-directed mutagenesis was performed using Phusion Flash high-fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), and the mutations were verified by sequencing. HEK293 cells were cultured in DMEM (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% FBS (Hyclone, Logan, UT, USA), and 2 mM glutamine, in a humidified atmosphere of 5% CO_2 at 37°C. Cells were plated on poly L-ornithine-coated glass coverslips and transiently transfected with 0.2 μg of the pcDNA3 containing hKir6.2 construct and 0.8 μg of pcDNA3 containing hSUR1 construct by using PolyJet (SignaGen, Rockville, MD, USA). Cells were used 2–4 days after transfection. K_{ATP} currents were recorded by an Axopatch 700B amplifier (Molecular Device, Sunnyvale, CA, USA) and data were acquired at 10 kHz with pClamp 10 software (Molecular Device, Sunnyvale, CA, USA). For whole-cell patch clamp recording, the extracellular solution consisted of 150 mM NaCl, 10 mM HEPES, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and pH 7.2, adjusted with NaOH. The intracellular solution containing: 135 mM K gluconate, 15 mM KCl, 10 mM HEPES, 0.5 mM Mg_2ATP, 1 mM Na_3GTP, 10 mM sodium phosphocreatine, 0.05 mM EGTA and pH 7.2, adjusted with KOH. For inside-out patch clamp recording, both the pipette and bath solution contained a high-potassium solution consisting of: 150 mM KCl, 10 mM HEPES, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and pH 7.2, adjusted with KOH. ATP (Sigma Aldrich, USA), ADP (Sigma Aldrich, USA) and diazoxide (Hello Bio, UK) were added to the bath solution as indicated. Pipettes were pulled from 1.5 mm borosilicate glass capillaries (Sutter, Novato, CA, USA). The access resistances ranged between 5 and 20 MΩ and were compensated by ~80%. All experiments were performed at room temperature (~25°C). Data were analyzed with pClamp10 software (Molecular Devices, Sunnyvale, CA, USA). Results are reported as mean ± SEM. Statistical analysis was performed using Prism 7 (GraphPad, La Jolla, CA, USA), with differences considered significant at $P < 0.05$. EC_{50} and IC_{50} of nucleotides or diazoxide on the K_{ATP} currents were calculated as described (25). For Western blot analysis, the K_{ATP} channel-transfected HEK293 cell lysate was run on SDS-

PAGE and transferred to a nitrocellulose membrane. The membrane was hybridized with a rabbit anti-SUR1 antibody (#PA5-103639, Thermo Fisher, USA), then incubated with horseradish peroxidase-conjugated secondary antibody (GE Healthcare, Little Chalfont, UK), and proteins were visualized by enhanced chemiluminescence (Super Signal West Femto, Pierce, IL, USA). β-actin was used as a loading control. For structure modeling, apo- (PDB: 6JB1) (26) and MgATP/MgADP-bound K_{ATP} channels (PDB: 6C3P) (6) were used as templates and visualized by PyMOL (http://www.pymol.org/).

3 Results

The present study included 13 patients (eight boys and five girls) suffering from diazoxide-unresponsive CHI. Clinical data are shown in Table 1. Patients' data were described previously (13), except for patients No.3 and 7. After extensive molecular analysis, pathogenic variants were identified in nine (69%) patients.

3.1 *ABCC8* variants

Seven different pathogenic variants of the *ABCC8* gene in five of the nine (56%) patients were identified. These pathogenic variants included five missense, one indel, and one splice site variants. The indel c.3124_3126delACCinsCAGCCAGGAACTG (p.T1042QfsX75) detected in three unrelated patients was the most common pathogenic variant identified in this study. All the three patients were compound heterozygotes, with the other pathogenic variants as p.R1393C, p.R1418C, and a novel c.1177-2A>G variant. Two of these patients inherited the indel variant from their unaffected mothers, while the third patient (patient No.7) received the indel from the unaffected father. The fourth patient was compound heterozygous for p.L366F and p.R797Q in the *ABCC8* gene and inherited from her unaffected parents, respectively. All these four patients had CHI diagnosed within one week after birth. Two (Patient No.3 and 7) suffered from hypoglycemic seizure before the diagnosis was confirmed. Because of a high demand of intravenous glucose supplement and poor response to high dose diazoxide treatment, all of them received a near-total pancreatectomy. The histological assessment showed diffuse islet cell hyperplasia in these four patients. One patient (patient No.1) developed diabetes mellitus immediately after pancreatectomy. Hypoglycemia persisted after pancreatectomy in the other three patients and was controlled by diazoxide only or in combination with octreotide.

In patient No.10, a single pathogenic variant (p.R74Q), located in the *ABCC8* gene and inherited from his father, was identified. The MLPA analysis did not detect deletions in the *ABCC8* gene. This patient presented with hypoglycemic seizure at the age of three month. CHI was diagnosed at the age of five month. He had focal adenomatous hyperplasia over the body of the pancreas. Euglycemia was achieved after resection of the focal lesion in this patient when he was 6 months old.

3.2 *KCNJ11* variants

We observed two pathogenic missense variants of *KCNJ11* in two of the nine (22%) patients. Both patients were in the heterozygous state and no additional genetic defect was detected by MLPA analysis. The first patient carried the p.E282K pathogenic variant of paternal origin. Her paternal history revealed no hypoglycemia in infancy or childhood, but diabetes mellitus was diagnosed in the 3rd decade of life and was controlled by an oral hypoglycemic agent. This patient, born at full term with a birth weight of 4,310 gram, presented with hypoglycemic seizure on the first day after birth. The diagnosis of CHI was confirmed soon after the onset of symptom. The second heterozygous patient carried the paternally inherited p.R34H variant in *KCNJ11* along with a rare missense variant c.853C>T (p.R285W) in *ABCC8*, which was categorized as a variant of unknown significance (VUS) in ClinVar. No hypoglycemia or diabetes mellitus was reported in paternal history. This patient also had macrosomia at birth with a birth body weight of 4,120 gram. She was noted to have hypoglycemic seizure at a later age when she was two months old and then CHI was diagnosed. Both two patients received a near-total pancreatectomy due to a high demand of intravenous glucose infusion to maintain euglycemia and unresponsiveness to high dose of diazoxide. The histological examination of the pancreas in both patients showed diffuse islet cell hyperplasia. They both needed octreotide to control hypoglycemia after pancreatectomy.

3.3 *GCK* variants

The *de novo* p.I211F mutation of *GCK* was identified in patient No.6 (Supplementary Figure 1A). He was born at full term with a birth weight of 5,840 grams and had hyperinsulinemic hypoglycemia that was diagnosed at the age of two days. Intravenous glucose infusion at a rate of 14 mg/kg/min was required to maintain euglycemia. The patient showed a poor response to diazoxide, and a near-total pancreatectomy was performed at the age of one month. Hypoglycemia persisted after surgery, but this condition was controlled with diazoxide and frequent feeding.

The pathogenic variant *GCK* c. 631A>T (p.I211F) was detected in 7 of 58 (12%) reads by WES of patient No.12 (Supplementary Figure 1C), which was failed to be detected by Sanger sequencing (Supplementary Figure 1B). She was born at full term with a birth weight of 4,300 grams. Several seizure episodes were documented at the age of 4 months. Hyperinsulinemic hypoglycemia was diagnosed at the age of 11 months. The patient needed glucose infusion at a rate as high as 25 mg/kg/min to maintain euglycemia. She did not respond to a high dose of diazoxide, and a near-total pancreatectomy was performed at the age of one year. The pathohistological examination of the pancreas revealed diffuse islet changes with multiple discrete intralobular islet cell hyperplasia. After surgery, the patient maintained euglycemia through therapy with diazoxide and octreotide.

TABLE 1 Clinical characteristics and genotype profiles of 13 patients with diazoxide-unresponsive congenital hyperinsulinism.

Patient No./Sex	GA(wks) /BBW (g)	Age at onset	Max GIR (mg/kg/min)	OP/ Histology	Post-OP medication	Mutation					
						Gene	Exon/ intron	Nucleotide change	Amino acid substitution	Parental origin	ACMG classification
1/M	38/5664	<1 d	25.3	NP/Dif	–	*ABCC8*	Exon 25	c.3124_3126delACCins CAGCCAGGAACTG	p.T1042QfsX75	Maternal	Pathogenic
							Exon 35	c.4252C>T	p.R1418C	Paternal	Likely Pathogenic
2/F	36/3960	<1 d	21.0	NP/Dif	D,O	*ABCC8*	Exon 7	c.1096C>T	p.L366F	Paternal	Uncertain Significance
							Exon 19	c.2390G>A	p.R797Q	Maternal	Uncertain Significance
3/F	36/4950	<1 d	20.0	NP/Dif	D,O	*ABCC8*	Intron 7	c.1177-2A>G	Aberrant splicing	Paternal	Pathogenic
							Exon 25	c.3124_3126delACCins CAGCCAGGAACTG	p.T1042QfsX75	Maternal	Pathogenic
4/F	39/4310	<1 d	23.0	NP/Dif	O	*KCNJ11*	Exon 1	c.844G>A	p.E282K	Paternal	Uncertain Significance
5/M	38/4100	1 d	24.0	NP/Dif	D	–	–	–	–	–	–
6/M	FT/5840	2 d	14.0	NP/Dif	D	*GCK*	Exon 6	c.631A>T	p.I211F	*De novo*	Likely Pathogenic
7/M	38/3380	3 d	19.0	NP/Dif	D	*ABCC8*	Exon 25	c.3124_3126delACCins CAGCCAGGAACTG	p.T1042QfsX75	Paternal	Pathogenic
							Exon 34	c.4177 C>T	p.R1393C	Maternal	Pathogenic
8/F	39/4120	2 m	11.3	NP/Dif	O	*KCNJ11*	Exon 1	c.101G>A	p.R34H	Paternal	Likely Pathogenic
9/M	40/3900	2 m	11.6	SP/Dif	–	–	–	–	–	–	–
10/M	39/3920	3 m	15.0	EN/Fo	–	*ABCC8*	Exon 2	c.221G>A	p.R74Q	Paternal	Likely Pathogenic
11/M	38/3670	4 m	13.0	NP/Dif	–	–	–	–	–	–	–
12/F	40/4300	4 m	25.0	NP/AtDif	D,O	*GCK**	Exon 6	c.631A>T	p.I211F	*De novo*	Likely Pathogenic
13/M	38/3400	8 m	8.0	NP/Dif	D	–	–	–	–	–	–

M, male; F, female; FT, full-term; d, day; m, month; max GIR, maximum glucose infusion rate; NP, near-total pancreatectomy; SP, subtotal pancreatectomy; EN, enucleation; Dif, diffuse; Fo, focal; AtDif, atypical diffuse; D, diazoxide; O, octreotide; ACMG, American College of Medical Genetics and Genomics.

*Identified as postzygotic mutation by whole exome sequencing.

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3.4 SNP haplotype analysis of the *ABCC8* gene

Two scales of haplotype regions containing the recurrent indel variant c.3124_3126delACC ins CAGCCAGGAACTG (rs786204542) were examined. Supplementary Table 2 displays the list of SNPs found within the test region. The haplotype configurations, consisting of 40-SNP combinations (comprising 20 SNPs on each side flanking the rs786204542 variant) are shown in Table 2. The haplotype frequency of "haplotype B" in 4062 controls was 3.53%, while the frequency in these three cases was 66.67%. All three tested cases carried at least one "haplotype B" allele. Based on our statistical analysis, it is unlikely that these three patients having the same rare variant rs786204542 coincidentally carried "haplotype B" (Fisher's exact *P* value = 2.28×10^{-5}; permutation *P* value < 10^{-6}).

The 100 Kb-SNP haplotypes, encompassing SNPs within bilateral 50 Kb regions flanking rs786204542 (a subset of the aforementioned 40-SNP haplotypes), are shown in Table 3. All these three tested cases were found to carry at least one "haplotype M" allele, which share the same allele composition as "haplotype B" within the overlapping region of the 40-SNP scenario. Similarly, the statistical analysis strongly disfavored the possibility of chance occurrences (Fisher's exact *P* value = 1.08×10^{-4}; permutation *P* value < 10^{-5}).

These findings provide robust evidence supporting the presence of rs786204542 within haplotype B/haplotype M, suggestive of a founder effect.

3.5 Functional characterization of K_{ATP} channel variants

We performed whole-cell recording to examine membrane currents. Weak-inwardly rectifying potassium current was observed in HEK293 cells transfected with wild-type K_{ATP} channels and dialyzed with MgATP (0.5 mM). The application of 300 μM diazoxide enhanced the current, whereas 5 μM glibenclamide inhibited it (Supplementary Figure 2B). In contrast, cells transfected with hSUR1-R797Q and hSUR1-R1393C K_{ATP} channels showed significantly reduced potassium currents and did not respond to K_{ATP} channel modulators (Supplementary Figures 2A, C, D).

It has been reported that the cell membrane K_{ATP} channel extensively glycosylated upon maturation is evidenced by an upper band on the Western blot (27). We observed this distinctive banding pattern in cells transfected with wild-type and hSUR1-L366F K_{ATP} channels, but not in cells with hSUR1-R797Q or hSUR1-R1393C variants (Figure 1A). These results suggest that

TABLE 2 Estimated single nucleotide polymorphism (SNP) haplotype frequency with statistics in the region of bilateral 20 SNPs flanking the indel variant rs786204542.

Haplotype Name	Haplotype SNP Composition[a]	Affected patients (n=3)		Controls (n=4062)		Fisher's exact test *P*-value[b]	Permutation *P*-value[c]
		Count	Percent (%)	Count	Percent (%)		
Haplotype A	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2-2-2-2-1	1	16.67	644	7.93	0.3911	
Haplotype B*	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2-2-2-2	4	66.67	287	3.53	2.28×10^{-5}	$< 10^{-6}$
Haplotype C	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-1-2-1-2-1-1-1-2-2-2-2-2-2-2-2-2-2-2-2-2-1	1	16.67	264	3.25	0.18036	
Haplotype D	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1	0	0	155	1.91		
Haplotype E	2-2-2-1-1-2-1-2-2-2-1-1-1-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2-2-2	0	0	62	0.76		
Haplotype F	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2	0	0	49	0.60		
Haplotype G	2-2-2-2-1-2-2-1-2-2-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2-2-2-2-2-1	0	0	48	0.59		
Haplotype H	2-2-2-2-1-2-2-1-2-2-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2	0	0	13	0.16		
Haplotype I	2-2-2-2-1-2-2-1-2-2-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2-2-2-2	0	0	7	0.09		
Haplotype J	2-2-2-1-1-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-1-1-2-2-1-2-2-2-1-2-1-2-2-2-2-2-2-2-2-2-2-2-1-2-2	0	0	2	0.02		

[a]SNP allele "2": major allele; SNP allele "1": minor allele.
[b]Comparisons of haplotype frequencies between affected patients and controls using Fisher's exact test.
[c]Comparison of frequencies of haplotype B between affected patients and controls using permutation test, permutation time=1000000.
*Common haplotype among affected patients.

TABLE 3 Estimated single nucleotide polymorphism (SNP) haplotype frequency with statistics in the region of bilateral 50 kb flanking the indel variant rs786204542.

Haplotype Name	Haplotype SNP Composition[a]	Affected patients (n=3)		Controls (n=4050)		Fisher's exact test *P*-value[b]	Permutation *P*-value[c]
		Count	Percent (%)	Count	Percent (%)		
Haplotype K	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2	1	16.67	879	10.85	0.4982	
Haplotype L	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2	0	0	541	6.68		
Haplotype M*	2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2	4	66.67	426	5.26	$1.08x10^{-4}$	$< 10^{-5}$
Haplotype N	2-2-2-2-2-2-2-2-2-1-2-1-2-1-2-1-1-1-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2	1	16.67	422	5.21	0.2751	
Haplotype O	2-1-1-1-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-1	0	0	60	0.74		
Haplotype P	2-2-2-2-2-2-2-2-2-1-1-2-2-1-2-2-2-2-1-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2	0	0	54	0.67		
Haplotype Q	2-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2	0	0	39	0.48		
Haplotype R	2-2-2-2-2-1-2-2-2-1-2-1-2-1-2-1-1-1-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2	0	0	25	0.31		
Haplotype S	2-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-1-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2-2	0	0	2	0.02		

[a]SNP allele "2": major allele; SNP allele "1": minor allele.
[b]Comparisons of haplotype frequencies between affected patients and controls using Fisher's exact test.
[c]Comparison of frequencies of haplotype M between affected offspring and controls using permutation test, permutation time=100000.
*Common haplotype among affected patients.

hSUR1-R797Q and hSUR1-R1393C mutant channels show trafficking defects.

The functional change in hSUR1-L366F K_{ATP} mutant channels was further examined using the inside-out patch clamp configuration technique. We found that HEK293 cells transfected with wild-type and mutant hSUR1-L366F K_{ATP} channels showed a typical K_{ATP} current that reversed at around 0 mV, the value of the potassium equilibrium potential under our experimental conditions (Figures 1B, C). A similar sensitivity to different intracellular ATP concentrations was observed in both wild-type and mutant hSUR1-L366F K_{ATP} channels (Figure 1D). However, hSUR1-L366F K_{ATP} channels were inhibited rather than potentiated by MgADP (Figures 1C, E), and more strikingly, diazoxide inhibited rather than activated hSUR1-L366F K_{ATP} channels (Figure 1F). These results suggest that hSUR1-L366F K_{ATP} channels have a gating defect. Leu366 is positioned at the interface between transmembrane domain 1 (TMD1) and transmembrane domain 2 (TMD2). When MgADP binds to the nucleotide binding domains formed along with the TMD1-TMD2 interface, it triggers conformational changes in SUR1 that subsequently activate the channel gate located at Kir6.2 (28). However, our structural modeling reveals that substituting Leu366 with the bulkier phenylalanine generates steric clashes with SUR1-L1288, thereby preventing the MgADP-induced conformational changes within the channel structure, as depicted in Figure 2.

4 Discussion

Two large-scale studies have reported that patients with CHI have a disease-causing variant detection rate ranging from 45.3% to 79%, and the detection rate of diazoxide-unresponsive CHI was approximately 90% (29, 30). In the present study, we identified pathogenic variants in nine of the 13 (69%) diazoxide-unresponsive patients with CHI. Patients with pathogenic variants in *ABCC8* and *KCNJ11* genes accounted for 78% (7/9) of variants detected. Our data confirm that pathogenic variants in the K_{ATP} channel-encoding genes are the most common underlying cause of diazoxide-unresponsive CHI in the Taiwanese patients studied, which is in accordance with that observed in cohorts of different ethnic backgrounds (29–35).

The indel c.3124_3126delACCinsCAGCCAGGAACTG (p.T1042QfsX75) of the *ABCC8* gene is a frameshift variant that results in a truncated SUR1 protein, which is believed to impair protein function (36). The p.T1042QfsX75 variant has been repeatedly detected in Chinese cohorts with CHI (32, 33, 37, 38), especially in patients from southern China (32, 33). In the present study, the detection of p.T1042QfsX75 in three unrelated patients suggests that this variant is a hotspot, and haplotype analysis supported that it is a founder variant in Taiwan. This may also explain the findings in southern China. One patient was compound heterozygous for the p.T1042QfsX75 indel variant and a novel

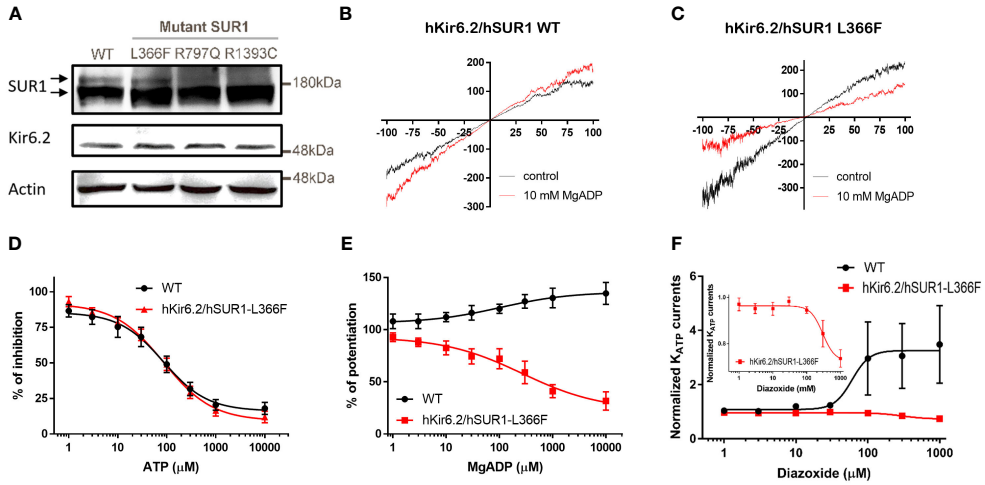

FIGURE 1
(A) Western blots show total SUR1 levels in HEK293 cells. SUR1 signals exhibit an upper band that corresponds to a fully glycosylated K_{ATP} channel (upper arrow), while the lower band represents immature K_{ATP} channels that are still stuck intracellularly (lower arrow) in wild-type (WT) SUR1. The upper bands are visible in HEK293 cells transfected with WT hSUR1 and hSUR1-L366F but absent in HEK293 cells transfected with hSUR1-R797Q and hSUR1-R1393C. β-actin serves as the loading control. **(B, C)** Representative voltage-clamp recording traces from inside-out patches containing K_{ATP} channels. Adding 10 mM MgADP (red traces) to the intracellular side potentiated WT hsSUR1 **(B)** but inhibited hSUR1-L366F **(C)** containing K_{ATP} currents. **(D)** WT and hSUR1-L366F containing K_{ATP} channels exhibited comparable sensitivity to the intracellular ATP (WT: IC_{50} = 85.4 μM, Hill slope = 0.97, n = 4; hSUR1-L366F: IC_{50} = 86.1 μM, Hill slope = 0.87, n = 6–8). **(E)** Intracellular MgADP potentiated WT K_{ATP} channels but inhibited hSUR1-L366F K_{ATP} (WT: EC_{50} = 102 μM, Hill slope = 0.65, n = 4–5; hSUR1-L366F: IC_{50} = 268 μM, Hill slope = 0.59, n = 3). **(F)** Diazoxide potentiates WT K_{ATP} Currents (EC_{50} = 60.1 μM, n = 5) but inhibits hSUR1-L366F K_{ATP} currents (IC_{50} = 297.7 μM, n = 4). The insert displays an enlarged dose-response curve of diazoxide on hSUR1-L366F K_{ATP} currents to highlight the inhibitory effect of diazoxide.

ABCC8 splice site variant, c.1177-2A>G. This splice site variant is predicted to disrupt the acceptor splice site of intron 7, resulting in the dysfunction of the protein. The other two patients were compound heterozygous for the indel and two different missense variants of the *ABCC8* gene, p.R1393C and p.R1418C, respectively. The SUR1 protein contains two nucleotide-binding domains (NBD1 and NBD2). Their dimerization is required for Mg^{2+}-dependent ATP hydrolysis (4, 39). These NBD domains are also required for channel regulation by MgADP and diazoxide (40, 41). Although the Arg1393 residue located in NBD2 is presumably involved in K_{ATP} channel gating, it has been reported that the p.R1393H causes a trafficking defect because mutant channels are retained in the trans-Golgi network (42). Our functional study confirmed that cysteine substitution at this position impairs the trafficking of the mutant channel to the surface of the plasma membrane. The *ABCC8* missense variant p.R1418C in a compound heterozygous state with a deletion of *ABCC8* exon 3 has been reported in a patient of diazoxide-unresponsive diffuse CHI (43). This missense variant is also located in NBD2 of SUR1. Although we did not evaluate the functional change of this variant, there is evidence showed histidine substitution at the Arg1418 residue impaired channel activity by confining the channel to the endoplasmic reticulum (ER) (44). The cysteine substitution at the same position is presumed to have a deleterious effect on channel function as well. Further study should be done to elucidate the underlying mechanism of the p.R1418C variant.

One patient was compound heterozygous for p.L366F and p.R797Q variants of the *ABCC8* gene. The p.L366F has been reported as a diazoxide-unresponsive dominant variant with phenotypes varying from asymptomatic to CHI or diabetes within family members (45). Our *in vitro* functional assays showed that hSUR1-L366F K_{ATP} mutant channels are inhibited rather than activated by intracellular MgADP, and the reversed effect of MgADP on K_{ATP} channel gating may negatively affect glucose-stimulated insulin secretion (GSIS). Under normal conditions, lowering plasma glucose would decrease the intracellular ATP/ADP ratio and activate K_{ATP} channels that silence beta cells and cease insulin secretion. In contrast, hSUR1-L366F K_{ATP} mutant channels can be inhibited by both ATP and ADP, remaining mostly closed even at low blood glucose levels, leading to altered insulin secretion. We observed that hSUR1-L366F K_{ATP} mutant channels are insensitive to diazoxide, supporting the clinical observation that patients with CHI carrying this variant show a diazoxide-unresponsive phenotype. The Leu366 residue is located in the first transmembrane domain of SUR1, above the sulfonylurea binding pocket (28). Our structural modeling indicated that Leu366 forms a hydrophobic interaction with Leu1288. A substitution of leucine to phenylalanine at this position would induce steric clashes with Leu1288, preventing conformation changes of SUR1 that propagate the MgADP signal from NBDs (6). Regarding the p.R797Q variant, it has been reported that p.R797W is a recessive pathogenic variant detected in patients with CHI (29). Our functional study demonstrated that glutamine substitution at this site impairs K_{ATP} channel trafficking.

In this study, three of seven (43%) patients carrying K_{ATP} variants were inherited from the paternal monoallelic mutations. The remaining four patients had biallelic pathogenic variants of the *ABCC8* gene and invariably exhibited a diazoxide-unresponsive

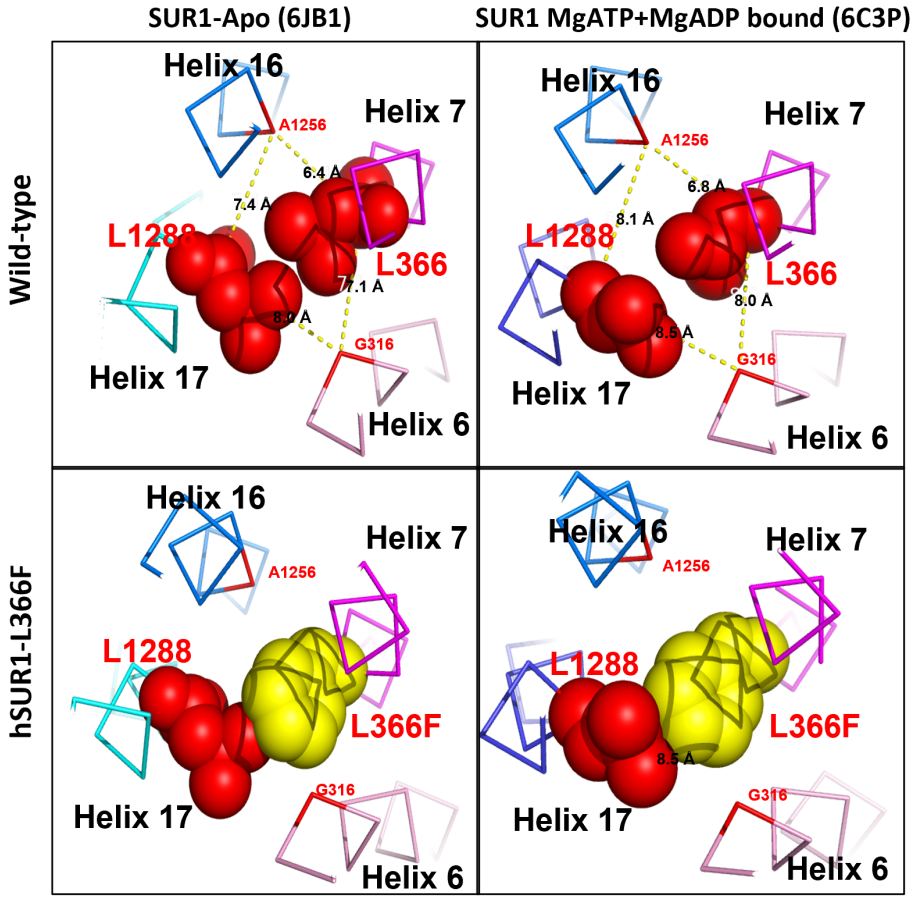

FIGURE 2
Structural prediction of SUR1 L366 and its interacting partner L1288 of K_{ATP} channel. In wild-type K_{ATP} channel, SUR1 L366 (red) could contact freely with its partner L1288 (red) via hydrophobic interaction in both Apo (PDB: 6JB1) and MgATP + MgADP bound form (PDB:6C3P) (upper panel). In contrast, in the hSUR1-L366F mutant K_{ATP} channel, the bulky phenylalanine (yellow) produces steric clashes against L1288 and consequently prevents the MgADP-induced conformational changes of SUR1 (lower panel).

diffuse CHI phenotype. Patients carrying paternally inherited monoallelic pathogenic variants in *ABCC8* or *KCNJ11* gene showed different clinical manifestations (46). A patient with the recessive p.R74Q variant in the *ABCC8* gene had focal CHI, which was cured after the excision of the pancreatic lesion. The histological assessment of two patients with paternally inherited heterozygous p.R34H and p.E282K variants in the *KCNJ11* gene revealed diffuse islet changes. A compound heterozygote of one missense variant p.R34H and one single-base deletion variant in *KCNJ11* has been reported in a patient with severe diazoxide-unresponsive CHI (34). It is less likely that the p.R34H variant in our patient acts in a dominant negative manner because the paternal carrier was clinically asymptomatic. As for the p.E282K variant, there is evidence showed that this variant abrogates the exit signal and prevents the ER export and surface expression of the channel when expressed alone. When co-expressed, the mutant subunit was able to associate with the wild-type Kir6.2 and form functional channels (47). Although a dominant effect on K_{ATP} channel function of this variant was suspected as revealed by the history of early onset type 2 diabetes mellitus in the paternal carrier, it suggests that the p.E282K variant acts recessively in our patient to cause loss of function of K_{ATP} channel and severe diazoxide-unresponsive phenotype. Patients with paternally acquired monoallelic recessive variants in the *KCNJ11* gene showed severe diffuse CHI, likely due to the unsuccessful attempts to detect cryptic pathogenic variants by current sequencing methods. Alternatively, patients may show an atypical diffuse form due to mosaic interstitial paternal uniparental isodisomy of chromosome 11p15.1 region (10).

Dominant gain-of-function mutations in the *GCK* gene are considered a rare cause of CHI, showing a detection rate lower than 1.7% (29–33, 35). Patients with CHI caused by mutations in the *GCK* gene exhibit intact K_{ATP} channels, supporting that potassium channel openers are effective for controlling hyperinsulinemic hypoglycemia (48), contrasting to those observed in our patients. The p. I211F mutation in the *GCK* gene led to a 12-fold higher $k_{cat}/K_{0.5,glucose}$ value than that of the wild-type glucokinase, resulting in a very low threshold for GSIS (49). Before the current study, a case of atypical diffuse CHI with a somatic mosaic p.I211F mutation in *GCK* has been reported (11, 50). Unlike our patient 6 who is heterozygous for this mutation, both the two patients with the somatic p.I211F mutation in *GCK* were less overweight at birth and

exhibited a more insidious onset of hypoglycemia after the neonatal period. However, these two patients eventually became diazoxide-unresponsive and needed pancreatectomy to control hypoglycemia. We speculate that the extent of β cell involvement of the mutant enzyme correlates with the severity of the initial clinical presentation in patients carrying p.I211F mutation in *GCK*, and all patients with this pathogenic variant are eventually prone to respond poorly to diazoxide treatment due to the highly active nature of the mutant enzyme. Our finding also supports somatic mutations in the *GCK* gene is a rare but important cause of atypical CHI.

In conclusion, we found that pathogenic variants in the K_{ATP} channel were the most common underlying cause of diazoxide-unresponsive CHI in thirteen Taiwanese children. We demonstrated that the high frequency of the p.T1042QfsX75 variant in the *ABCC8* gene is likely due to a founder effect in Taiwan. Our study demonstrated that patients carrying three rare SUR1 mutants with diazoxide-unresponsive phenotype are due to K_{ATP} channel gating defect (p.L366F) or trafficking defect (p.R797Q and p.R1393C). Our study also confirmed that the p.I211F variant of the *GCK* gene results in diazoxide-unresponsive CHI. Additional management, including near-total pancreatectomy, may be required to maintain euglycemia in patients with the p.I211F variant.

Data availability statement

The data presented in the study are deposited in the DNA Data Bank of Japan (DDBJ) repository, accession number LC785398, LC785399, LC785400, LC785401, LC785402, LC785403, LC785404, LC785405, LC785406, LC785407.

Ethics statement

The studies involving humans were approved by Research Ethics Committee of the National Taiwan University Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

C-TL: Funding acquisition, Investigation, Writing – original draft, Writing – review & editing. W-HT: Investigation, Visualization, Writing – review & editing. C-CC: Formal analysis, Writing – original draft, Writing – review & editing. P-CChen: Investigation, Writing – review & editing. CF: Formal analysis, Writing – review & editing. H-KC: Investigation, Visualization, Writing – review & editing. S-YL: Resources, Writing – review & editing. M-ZW: Investigation, Writing – review & editing. P-CChiu: Resources, Writing – review & editing. W-MH: Resources, Writing – review & editing. W-SY: Conceptualization, Supervision, Writing – review & editing. L-PL: Supervision, Writing – review & editing. W-YT: Conceptualization, Supervision, Writing – review & editing. S-BY: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing. P-LC: Conceptualization, Supervision, Writing – review & editing.

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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.2023.1283907/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Sequencing results of mutation found in the *GCK* gene.

SUPPLEMENTARY FIGURE 2
Whole-cell patch clamp recordings of HEK293 cells transfected with hSUR1-R797Q and hSUR1-R1393C K_{ATP} channels.

References

1. Menni F, de Lonlay P, Sevin C, Touati G, Peigne 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

2. 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

3. Ashcroft FM. ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest* (2005) 115(8):2047–58. doi: 10.1172/JCI25495

4. Shyng S, Ferrigni T, Nichols CG. Regulation of KATP channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. *J Gen Physiol* (1997) 110(6):643–54. doi: 10.1085/jgp.110.6.643

5. Baukrowitz T, Schulte U, Oliver D, Herlitze S, Krauter T, Tucker SJ, et al. PIP2 and PIP as determinants for ATP inhibition of KATP channels. *Science* (1998) 282 (5391):1141–4. doi: 10.1126/science.282.5391.1141

6. Lee KPK, Chen J, Mackinnon R. Molecular structure of human KATP in complex with ATP and ADP. *eLife* (2017) 6:e32481. doi: 10.7554/eLife.32481

7. 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

8. 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(6):2177–84. doi: 10.1016/S0002-9440(10)64689-5

9. 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. *New Engl J Med* (1999) 340(15):1169–75. doi: 10.1056/NEJM199904153401505

10. Hussain K, Flanagan SE, Smith VV, Ashworth M, Day M, Pierro A, et al. An ABCC8 gene mutation and mosaic uniparental isodisomy resulting in atypical diffuse congenital hyperinsulinism. *Diabetes* (2008) 57(1):259–63. doi: 10.2337/db07-0998

11. Sempoux C, Capito C, Bellanne-Chantelot C, Verkarre V, de Lonlay P, Aigrain Y, et al. Morphological mosaicism of the pancreatic islets: a novel anatomopathological form of persistent hyperinsulinemic hypoglycemia of infancy. *J Clin Endocrinol Metab* (2011) 96(12):3785–93. doi: 10.1210/jc.2010-3032

12. Aynsley-Green A, Hussain K, Hall J, Saudubray JM, Nihoul-Fekete C, De Lonlay-Debeney P, et al. Practical management of hyperinsulinism in infancy. *Arch Dis Childhood Fetal Neonatal Edition* (2000) 82(2):F98–F107. doi: 10.1136/fn.82.2.f98

13. Lee CT, Liu SY, Tung YC, Chiu PC, Wu MZ, Tsai WY. Clinical characteristics and long-term outcome of Taiwanese children with congenital hyperinsulinism. *J Formosan Med Assoc* (2016) 115(5):306–10. doi: 10.1016/j.jfma.2015.04.002

14. de Lonlay P, Fournet JC, Touati G, Groos MS, Martin D, Sevin C, et al. Heterogeneity of persistent hyperinsulinaemic hypoglycaemia. A series of 175 cases. *Eur J Pediatr* (2002) 161(1):37–48. doi: 10.1007/s004310100847

15. 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(5):405–24. doi: 10.1038/gim.2015.30

16. Chen MJ, Wei SY, Yang WS, Wu TT, Li HY, Ho HN, et al. Concurrent exome-targeted next-generation sequencing and single nucleotide polymorphism array to identify the causative genetic aberrations of isolated Mayer-Rokitansky-Kuster-Hauser syndrome. *Hum Reprod* (2015) 30(7):1732–42. doi: 10.1093/humrep/dev095

17. Zhang H, Colclough K, Gloyn AL, Pollin TI. Monogenic diabetes: a gateway to precision medicine in diabetes. *J Clin Invest* (2021) 131(3):e142244. doi: 10.1172/JCI142244

18. Wei CY, Yang JH, Yeh EC, Tsai MF, Kao HJ, Lo CZ, et al. Genetic profiles of 103,106 individuals in the Taiwan Biobank provide insights into the health and history of Han Chinese. *NPJ Genomic Med* (2021) 6(1):10. doi: 10.1038/s41525-021-00178-9

19. Saeed S, Mahjabeen I, Sarwar R, Bashir K, Kayani MA. Haplotype analysis of XRCC2 gene polymorphisms and association with increased risk of head and neck cancer. *Sci Rep* (2017) 7(1):13210. doi: 10.1038/s41598-017-13461-6

20. Liu E, Percy MJ, Amos CI, Guan Y, Shete S, Stockton DW, et al. The worldwide distribution of the VHL 598C>T mutation indicates a single founding event. *Blood* (2004) 103(5):1937–40. doi: 10.1182/blood-2003-07-2550

21. Cornes BK, Tang CS, Leon TY, Hui KJ, So MT, Miao X, et al. Haplotype analysis reveals a possible founder effect of RET mutation R114H for Hirschsprung's disease in the Chinese population. *PloS One* (2010) 5(6):e10918. doi: 10.1371/journal.pone.0010918

22. Lin YC, Hsieh AR, Hsiao CL, Wu SJ, Wang HM, Lian Ie B, et al. Identifying rare and common disease associated variants in genomic data using Parkinson's disease as a model. *J Biomed Sci* (2014) 21(1):88. doi: 10.1186/s12929-014-0088-9

23. Yang YY, Long RK, Ferrara CT, Gitelman SE, German MS, Yang SB. A new familial form of a late-onset, persistent hyperinsulinemic hypoglycemia of infancy caused by a novel mutation in KCNJ11. *Channels* (2017) 11(6):636–47. doi: 10.1080/19336950.2017.1393131

24. Lin CH, Lin YC, Yang SB, Chen PC. Carbamazepine promotes surface expression of mutant Kir6.2-A28V ATP-sensitive potassium channels by modulating Golgi retention and autophagy. *J Biol Chem* (2022) 298(5):101904. doi: 10.1016/j.jbc.2022.101904

25. Yang SB, Major F, Tietze LF, Rupnik M. Block of delayed-rectifier potassium channels by reduced haloperidol and related compounds in mouse cortical neurons. *J Pharmacol Exp Ther* (2005) 315(1):352–62. doi: 10.1124/jpet.105.086561

26. Ding D, Wang M, Wu J-X, Kang Y, Chen L. The structural basis for the binding of repaglinide to the pancreatic KATP channel. *Cell Rep* (2019) 27(6):1848–1857.e4. doi: 10.1016/j.celrep.2019.04.050

27. Chen PC, Olson EM, Zhou Q, Kryukova Y, Sampson HM, Thomas DY, et al. Carbamazepine as a novel small molecule corrector of trafficking-impaired ATP-sensitive potassium channels identified in congenital hyperinsulinism. *J Biol Chem* (2013) 288(29):20942–54. doi: 10.1074/jbc.M113.470948

28. Puljung MC. Cryo-electron microscopy structures and progress toward a dynamic understanding of K(ATP) channels. *J Gen Physiol* (2018) 150(5):653–69. doi: 10.1085/jgp.201711978

29. 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):E355–363. doi: 10.1210/jc.2012-2169

30. 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

31. Sang Y, Xu Z, Liu M, Yan J, Wu Y, Zhu C, et al. Mutational analysis of ABCC8, KCNJ11, GLUD1, HNF4A and GCK genes in 30 Chinese patients with congenital hyperinsulinism. *Endocrine J* (2014) 61(9):901–10. doi: 10.1507/endocrj.ej13-0398

32. Ni J, Ge J, Zhang M, Hussain K, Guan Y, Cheng R, et al. Genotype and phenotype analysis of a cohort of patients with congenital hyperinsulinism based on DOPA-PET CT scanning. *Eur J Pediatr* (2019) 178(8):1161–9. doi: 10.1007/s00431-019-03408-6

33. Xu A, Cheng J, Sheng H, Wen Z, Lin Y, Zhou Z, et al. Clinical management and gene mutation analysis of children with congenital hyperinsulinism in south China. *J Clin Res Pediatr Endocrinol* (2019) 11(4):400–9. doi: 10.4274/jcrpe.galenos.2019.2019.0046

34. Ohkubo K, Nagashima M, Naito Y, Taguchi T, Suita S, Okamoto N, et al. Genotypes of the pancreatic beta-cell K-ATP channel and clinical phenotypes of Japanese patients with persistent hyperinsulinaemic hypoglycaemia of infancy. *Clin Endocrinol* (2005) 62(4):458–65. doi: 10.1111/j.1365-2265.2005.02242.x

35. Park SE, Flanagan SE, Hussain K, Ellard S, Shin CH, Yang SW. Characterization of ABCC8 and KCNJ11 gene mutations and phenotypes in Korean patients with congenital hyperinsulinism. *Eur J Endocrinol* (2011) 164(6):919–26. doi: 10.1530/EJE-11-0160

36. Ellard S, Flanagan SE, Girard CA, Patch AM, Harries LW, Parrish A, et al. Permanent neonatal diabetes caused by dominant, recessive, or compound heterozygous SUR1 mutations with opposite functional effects. *Am J Hum Genet* (2007) 81(2):375–82. doi: 10.1086/519174

37. Fan ZC, Ni JW, Yang L, Hu LY, Ma SM, Mei M, et al. Uncovering the molecular pathogenesis of congenital hyperinsulinism by panel gene sequencing in 32 Chinese patients. *Mol Genet Genomic Med* (2015) 3(6):526–36. doi: 10.1002/mgg3.162

38. Xu ZD, Hui PP, Zhang W, Zeng Q, Zhang L, Liu M, et al. Analysis of clinical and genetic characteristics of Chinese children with congenital hyperinsulinemia that is spontaneously relieved. *Endocrine* (2021) 72(1):116–23. doi: 10.1007/s12020-020-02585-x

39. Zhao C, MacKinnon R. Molecular structure of an open human KATP channel. *Proc Natl Acad Sci United States America* (2021) 118(48):e2112267118. doi: 10.1073/pnas.2112267118

40. Martin GM, Yoshioka C, Rex EA, Fay JF, Xie Q, Whorton MR, et al. Cryo-EM structure of the ATP-sensitive potassium channel illuminates mechanisms of assembly and gating. *eLife* (2017) 6:e24149. doi: 10.7554/eLife.24149

41. Wang M, Wu J-X, Ding D, Chen L. Structural insights into the mechanism of pancreatic KATP channel regulation by nucleotides. *Nat Commun* (2022) 13(1):2770. doi: 10.1038/s41467-022-30430-4

42. Partridge CJ, Beech DJ, Sivaprasadarao A. Identification and pharmacological correction of a membrane trafficking defect associated with a mutation in the sulfonylurea receptor causing familial hyperinsulinism. *J Biol Chem* (2001) 276 (38):35947–52. doi: 10.1074/jbc.M104762200

43. Khawash P, Hussain K, Flanagan SE, Chatterjee S, Basak D. Nifedipine in congenital hyperinsulinism - A case report. *J Clin Res Pediatr Endocrinol* (2015) 7 (2):151–4. doi: 10.4274/jcrpe.1978

44. Tornovsky S, Crane A, Cosgrove KE, Hussain K, Lavie J, Heyman M, et al. Hyperinsulinism of infancy: novel ABCC8 and KCNJ11 mutations and evidence for additional locus heterogeneity. *J Clin Endocrinol Metab* (2004) 89(12):6224–34. doi: 10.1210/jc.2004-1233

45. Rasmussen AG, Melikian M, Globa E, Detlefsen S, Rasmussen L, Petersen H, et al. The difficult management of persistent, non-focal congenital hyperinsulinism: a retrospective review from a single, tertiary center. *Pediatr Diabetes* (2020) 21(3):441–55. doi: 10.1111/pedi.12989

46. Arya VB, Guemes M, Nessa A, Alam S, Shah P, Gilbert C, et al. Clinical and histological heterogeneity of congenital hyperinsulinism due to paternally inherited heterozygous ABCC8/KCNJ11 mutations. *Eur J Endocrinol* (2014) 171(6):685–95. doi: 10.1530/EJE-14-0353

47. Taneja TK, Mankouri J, Karnik R, Kannan S, Smith AJ, Munsey T, et al. Sar1-GTPase-dependent ER exit of KATP channels revealed by a mutation causing congenital hyperinsulinism. *Hum Mol Genet* (2009) 18(13):2400–13. doi: 10.1093/hmg/ddp179

48. Glaser B, Kesavan P, Heyman M, Davis E, Cuesta A, Buchs A, et al. Familial hyperinsulinism caused by an activating glucokinase mutation. *New Engl J Med* (1998) 338(4):226–30. doi: 10.1056/NEJM199801223380404

49. Pal P, Miller BG. Activating mutations in the human glucokinase gene revealed by genetic selection. *Biochemistry* (2009) 48(5):814–6. doi: 10.1021/bi802142q

50. Henquin JC, Sempoux C, Marchandise J, Godecharles S, Guiot Y, Nenquin M, et al. Congenital hyperinsulinism caused by hexokinase I expression or glucokinase-activating mutation in a subset of beta-cells. *Diabetes* (2013) 62:1689–96. doi: 10.2337/db12-1414

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Hypoglycaemia in adrenal insufficiency

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Adrenal insufficiency encompasses a group of congenital and acquired disorders that lead to inadequate steroid production by the adrenal glands, mainly glucocorticoids, mineralocorticoids and androgens. These may be associated with other hormone deficiencies. Adrenal insufficiency may be primary, affecting the adrenal gland's ability to produce cortisol directly; secondary, affecting the pituitary gland's ability to produce adrenocorticotrophic hormone (ACTH); or tertiary, affecting corticotrophin-releasing hormone (CRH) production at the level of the hypothalamus. Congenital causes of adrenal insufficiency include the subtypes of Congenital Adrenal Hyperplasia, Adrenal Hypoplasia, genetic causes of Isolated ACTH deficiency or Combined Pituitary Hormone Deficiencies, usually caused by mutations in essential transcription factors. The most commonly inherited primary cause of adrenal insufficiency is Congenital Adrenal Hyperplasia due to 21-hydroxylase deficiency; with the classical form affecting 1 in 10,000 to 15,000 cases per year. Acquired causes of adrenal insufficiency can be subtyped into autoimmune (Addison's Disease), traumatic (including haemorrhage or infarction), infective (e.g. Tuberculosis), infiltrative (e.g. neuroblastoma) and iatrogenic. Iatrogenic acquired causes include the use of prolonged exogenous steroids and post-surgical causes, such as the excision of a hypothalamic-pituitary tumour or adrenalectomy. Clinical features of adrenal insufficiency vary with age and with aetiology. They are often non-specific and may sometimes become apparent only in times of illness. Features range from those related to hypoglycaemia such as drowsiness, collapse, jitteriness, hypothermia and seizures. Features may also include signs of hypotension such as significant electrolyte imbalances and shock. Recognition of hypoglycaemia as a symptom of adrenal insufficiency is important to prevent treatable causes of sudden deaths. Cortisol has a key role in glucose homeostasis, particularly in the counter-regulatory mechanisms to prevent hypoglycaemia in times of biological stress. Affected neonates particularly appear susceptible to the compromise of these counter-regulatory mechanisms but it is recognised that affected older children and adults remain at risk of hypoglycaemia. In this review, we summarise the pathogenesis of hypoglycaemia in the context of adrenal insufficiency. We further explore the clinical features of hypoglycaemia based on different age groups and the burden of the disease, focusing on hypoglycaemic-related events in the various

aetiologies of adrenal insufficiency. Finally, we sum up strategies from published literature for improved recognition and early prevention of hypoglycaemia in adrenal insufficiency, such as the use of continuous glucose monitoring or modifying glucocorticoid replacement.

KEYWORDS

hypoglycaemia, adrenal insufficiency, hypoadrenalism, cortisol, glucocorticoid

1 Introduction

The adrenal glands are responsible for the production of 3 main steroids, mineralocorticoids (mainly aldosterone), glucocorticoids (mainly cortisol) and androgens. These are produced by the outer zona glomerulosa, middle zona fasciculata and inner zona reticularis of the adrenal cortex respectively. Cortisol and androgen productions are influenced by the hypothalamic-pituitary axis, whilst aldosterone is regulated by the renin-angiotensin system. Adrenal insufficiency (AI) occurs when there is primary adrenal failure or disruption to the hypothalamic-pituitary axis leading to inadequate steroid secretion. AI has different clinical presentations depending on the age and cause. Newborns with AI typically present with severe hypoglycaemia, seizures, failure to thrive, prolonged cholestatic jaundice, and in some cases coma. The lack of cortisol results in slow transport and maturation of bile acid synthesis, causing conjugated hyperbilirubinemia with raised liver enzymes, which usually presents at a median age of 13 days of life (1). In children and young adults with AI, they may have hypoglycaemia, weakness, fatigue, gastrointestinal symptoms, headaches, muscle and joint pains (1, 2). Symptoms may sometimes be non-specific such as postural hypotension, syncope, arthralgia, anorexia and mental health issues, as described in a case report of undiagnosed Addison's disease who presented to the hospital in a collapsed state (3). Individuals with secondary adrenal insufficiency (SAI) due to abnormalities in the pituitary gland and hypothalamus may exhibit symptoms involving other hormone deficiencies or have coinciding midline defects. The risk of hypoglycaemia is increased if they have accompanying growth hormone (GH) deficiency, due to the counterregulatory role of GH in hypoglycaemia which is discussed later in this paper.

Adrenal crisis is a life-threatening complication of AI due to the body's inability to respond to physiological stress. It is more common in PAI (primary adrenal insufficiency) compared to SAI (4, 5). Symptoms include hypotension, dehydration, vomiting, abdominal pain, and in the most serious cases, they may present with shock, coma and death (6). Unexplained sudden deaths in neonates and children should always raise the suspicion of an adrenal crisis. Data looking at hypoglycaemia-related deaths in patients with PAI is limited, however, there are published studies examining the effect of hypoglycaemia on the mortality rates of patients with SAI who are on growth hormones (7–9). In a large cohort study of patients who received growth hormone in the United States, 24.5% (106 out of the 433) deaths recorded were found to be sudden and unexpected. 74% of these unexpected deaths were associated with multiple pituitary hormone deficiencies and hypoglycaemia was highlighted in 31% of these 106 deaths. In addition, more than half of the unexpected deaths were thought to have undiagnosed secondary adrenal insufficiency (9).

Individuals with AI are at a higher risk of a hypoglycaemic event, especially when unwell or in an adrenal crisis. Hypoglycaemia can have serious consequences if left unrecognised and untreated. We aim to critically assess the burden of hypoglycaemia in the population of adrenal insufficiency and investigate ways to reduce morbidity and mortality in children with AI. We performed a literature search in Pubmed using the terms 'primary adrenal insufficiency', 'secondary adrenal insufficiency', 'cortisol deficiency', 'hypoadrenalism', 'adrenal crises', 'hypoglycaemia' and 'low blood sugars'. Due to lack of reliable available evidence, we also included specific conditions known to cause AI for a more comprehensive review- mainly congenital adrenal hyperplasia (CAH), congenital adrenal hypoplasia, panhypopituitarism and Addison's disease. All papers related to the search terms above were included in this review. In this manuscript, we have explored the counterregulatory mechanism of hypoglycaemia, reasons for individuals with AI to be more susceptible to hypoglycaemia, their clinical presentation and potential complications, current management and future developments.

2 Counterregulatory mechanism of hypoglycaemia

Under normal circumstances, when blood glucose falls below the physiological range, insulin production decreases and counterregulatory hormones are released to maintain glucose supply for vital bodily functions. The initial counterregulatory hormones released include glucagon and epinephrine. Both glucagon and epinephrine act by stimulating hepatic glycogenolysis and promoting gluconeogenesis. Epinephrine also limits glucose clearance by insulin-sensitive tissues (10).

Other counterregulatory hormones such as growth hormone and cortisol are secreted as blood glucose continues to fall. Both these hormones combat hypoglycaemia over a longer time frame. The role of growth hormone and cortisol in regulating the severity

of hypoglycaemia during sustained intravenous insulin infusion was demonstrated in two studies by De Feo et. al (11, 12). Growth hormone and cortisol induce lipolysis in fat tissues and encourage hepatic ketogenesis and gluconeogenesis (13). Lipolysis causes an increase in free fatty acids which affects insulin signalling pathways (14). Cortisol also reduces insulin secretion from the pancreas (15). In glucose homeostasis, the rise of glucose leads to an increase in insulin production. Growth hormone also stimulates the production of insulin-like growth factor 1 (IGF-1) from the liver, provided that there is adequate nutrition and elevated portal insulin levels (16). Normoglycaemia is achieved as glucose is absorbed back into peripheral cells.

3 Hypoglycaemia in adrenal insufficiency

3.1 Aetiology

In general, children and young infants tend to be more vulnerable to the effects of hypoglycaemia. Neonatal hypoglycaemia can affect neurodevelopmental outcomes as neonates have higher brain glucose requirements, coupled with an immature pathway to respond to low blood sugars. Children have higher energy demand for growth and reduced glycogen supply compared to adults (17). Steady control of blood glucose is vital for neurocognitive development in children. A meta-analysis of children with Type 1 diabetes showed lower capabilities in memory and learning in those who had recurrent severe hypoglycaemic episodes compared to those who did not (18).

A few theories have been proposed to explain why individuals with AI are more susceptible to hypoglycaemia. Excess cortisol has been linked with an increase in insulin resistance (19, 20), possibly due to a rise in circulating fatty acids from cortisol-induced lipolysis (21, 22). Lack of cortisol has been shown to increase insulin sensitivity (23), which will enhance peripheral tissue's uptake of glucose, thereby increasing the risk of a hypoglycaemia event. In addition, cortisol deficiency causes a reduction in endogenous glucose production and an increase in glucose oxidation, which can lead to hypoglycaemia (23). An impaired physiological rise in blood glucose levels during exercise was also seen in patients with classical CAH, even when they have good compliance with their glucocorticoid treatment. Doubling the hydrocortisone dose did not affect the exercise glycaemic levels in these cases (24).

Normal glucocorticoid secretion of the adrenal cortex is essential for optimal development and functioning of the adrenal medulla, which is responsible for the production of the majority of epinephrine hormone (25). Patients with CAH were found to have reduced epinephrine stores which may affect their counter-regulatory mechanism towards hypoglycaemia (26). A separate study has shown poorly developed adrenomedullary structures and fewer secretory vesicles in the chromaffin cells of patients with congenital adrenal hyperplasia (CAH) (27). Patients with AI are also more sensitive to hypoglycaemia due to the lack of epinephrine response during a hypoglycaemic event (28). Blunted epinephrine response was seen in all patients with pituitary adenoma during insulin-induced hypoglycaemia, but the impairment is worst in those patients with SAI (29).

Hypoglycaemia risk in children with SAI is dependent on the cause and presence of associated hormone deficiencies. In a case series, 3 children with a background of asthma who were on a high dose of inhaled corticosteroids, were found to have SAI after presenting with hypoglycaemia, accompanied by vomiting, drowsiness and tiredness. They suggested that adrenal suppression is influenced by patient susceptibility, dose and duration of the inhaled corticosteroids (30). Low cortisol levels during controlled hypoglycaemia in neonates with hyperinsulinism were reported in a clinical trial. This study demonstrated that hypocortisolaemia was due to inappropriately low ACTH levels, indicating that neonates with hyperinsulinism may have blunted ACTH response from the hypothalamic-pituitary axis during a hypoglycaemia event (31).

3.2 Clinical presentation and complications of hypoglycaemia in AI

Patients with AI are always at risk of an adrenal crisis. The incidence of adrenal crises in individuals with primary or secondary AI is approximately 5-10 per 100 patient-years (4, 5, 32). However, this incidence is higher in PAI (33). Reisch et al. reported more than 70% of these events occurred in the first 10 years of life in children with 21-hydroxylase deficiency (34). In a study following children with CAH up to 6 years of age, the number of adrenal crises is roughly 6.5 per 100 patient-years (35).

The frequency of hypoglycaemia during adrenal crises is not widely studied. In the adult population with AI, hypoglycaemia is less common. A study in Japan diagnosed adrenal insufficiency in 32 out of 528 patients (6%) after presenting to the emergency department with hypoglycaemia. Their symptoms include tremors, palpitations, sweating, hunger, paresthesia, dizziness, weakness, and confusion (36). Adult patients may also exhibit mild symptoms such as early morning headaches due to unrecognised nocturnal hypoglycaemia (37).

In children, a study conducted in France reported 30 out of 165 (18%) children and infants with PAI experienced hypoglycaemic episodes. Those who experienced hypoglycaemia had a significantly lower basal cortisol level. Additionally, all of these hypoglycaemic episodes were associated with prior fasting or poor oral intake due to vomiting or viral illness (38). In a study looking at emergency hospital admissions of children with CAH, 24 admissions (9%) were due to hypoglycaemia and a third of these presented with hypoglycaemic seizures (39). Pinto et al. reported about 8% of complications in CAH children were due to hypoglycaemia and these occurred between the ages of 1 and 3 years old (40). Besides that, a small study monitoring acute illness in children with CAH documented 3 out of 8 occasions of illness were associated with hypoglycaemia confirmed by capillary blood glucose monitoring. They all had lethargy as a common symptom (41).

In a cohort study following children with CAH up to their 6th year of life, hypoglycaemia episodes were recorded in 16 out of 102

children (15%). There were a total of 23 hypoglycaemic episodes and 7 of these episodes were associated with salt loss. Some of these children had more than one hypoglycaemic episode, suggesting varying individuals' predispositions to hypoglycaemia. Loss of consciousness was seen in 13 out of the 16 hypoglycaemic episodes, and 5 children had hypoglycaemic seizures (35). In one study, 8 out of 63 (12%) children with Addison's disease in a rural and urban Pakistan province presented with hypoglycaemia during an adrenal crisis (42).

One published cohort study in the UK from 1987 to 2017 showed a significant rise in mortality rates in patients with AI compared to matched healthy controls, and the risk is significantly higher in patients with PAI compared to SAI. Adrenal crisis was found to be an associated cause of death in 10% of those with adrenal insufficiency, and this was mainly due to a compromise in the circulatory system. Although it was difficult to ascertain if hypoglycaemia partly contributed to the adrenal crisis-associated deaths, it was identified that mortality rates in patients with AI and accompanying diabetes were higher than those with AI alone (43). Data from patients with CAH showed mortality rates in developed countries vary between 0 to 4% (44). One study calculated the standardized mortality ratio (SMR) to be 3 times higher in children with CAH, and the mortality was significantly higher in infancy and children up to 4 years old (45), especially in the salt-wasting phenotype (46, 47). An extensive 30-year study found a reduction in deaths from neonatal salt-wasting crises in the second half of their observation period, suggesting that there may be an improved understanding of the emergency management of CAH (46). It remains challenging to extrapolate if hypoglycaemia played a role in these salt-wasting crises. However, we found one other study that reported 2 deaths in young children with CAH from hypoglycaemic seizures (40).

3.3 Treatment and prevention

AI treatment aims to mimic the circadian rhythm, and in neonates, this is not fully established until about 3 months of age (48). The basal secretion of cortisol in children and young adults is known to be between 5-6mg/m2/day (1). Due to gastric acids and hepatic first-pass effect, the recommended glucocorticoid treatment in children with primary AI is slightly higher, ranging between 6-12mg/m2/day (1, 49). Children with secondary AI may require a lower daily replacement dose (6-8mg/m2/day) (49). An optimal balance of glucocorticoid regimen is vital as patients with AI will require lifelong steroid replacement. Overreplacement of glucocorticoids has been associated with increased mortality (50), and under-replacement may lead to adrenal crises and hypoglycaemia. One prospective 2-year study has closely monitored and titrated the doses of hydrocortisone based on salivary 17-OHP levels in children with CAH up to 8 years of age and the outcome was a lower median daily hydrocortisone dose with no observed adrenal crises (51). This stresses the importance of regularly monitoring all patients with AI on steroid replacement.

Hydrocortisone is the preferred glucocorticoid and it is usually given in 3 divided doses, with a bigger dose in the morning aiming to mimic the physiological circadian rhythm (1). However, this is challenging to achieve as the circadian cortisol level will usually start to rise in the early hours of the morning from 0200. Current conventional immediate-release hydrocortisone treatment is unable able to mimic this (49). Insulin sensitivity is also the highest between 0200 and 0400, causing patients with AI to be at risk of nocturnal hypoglycaemia (52).

During an adrenal crisis, emergency hydrocortisone needs to be given immediately and this can be done via intramuscular injection (IM) or intravenously (IV). The doses of emergency hydrocortisone treatment are summarised in the above Table 1 in keeping with the latest British Society for Paediatric Endocrinology and Diabetes (BSPED) guideline. If the child is hypoglycaemic but conscious, fast-acting carbohydrates such as sugary juice should be given, followed by long-acting carbohydrates such as biscuits. If the child is unconscious, a 2ml/kg fluid bolus of 10% dextrose should be given and the blood sugar should be repeated in 10 to 15 minutes. Repeat dextrose fluid boluses may be given if necessary. Administration of intramuscular glucagon could be considered if healthcare professionals are unable to obtain intravenous access. The emergency management of hypoglycaemia is outlined in Figure 1 below. If a child with known AI is found to be hypoglycaemic and unconscious in the community, it is recommended that both intramuscular hydrocortisone and glucagon injections are administered immediately prior to arrival at the hospital.

The mainstay of early recognition and prevention of hypoglycaemia in adrenal insufficiency has been parental education. Children with AI are prone to hypoglycaemic events if they are not compliant with their steroid replacement (53). It is also important to educate parents/carers and older children with adrenal insufficiency on hydrocortisone stress dosing during an illness or injury (5). A National Patient Safety Alert has been issued in the UK for an emergency steroid card for all patients with AI, detailing clear instructions on what to do in an event of a suspected adrenal crisis. Despite these measures, a review study has found that parental

TABLE 1 Emergency hydrocortisone doses as per BSPED 2023 guidelines.

Age of Child	Community	Hospital
Neonates (< 28 days)	25mg IM dose	4mg/kg IV initially 4–6 hourly Once stable: 2mg/kg IV 4–6 hourly
Children (< 1 year) Children (1–5 years) Children (> 6 years)	25mg IM dose 50mg IM dose 100mg IM dose	2mg/kg (max 100mg) IV initially 4–6 hourly Once stable: 1mg/kg (max 50mg) IV 4–6 hourly

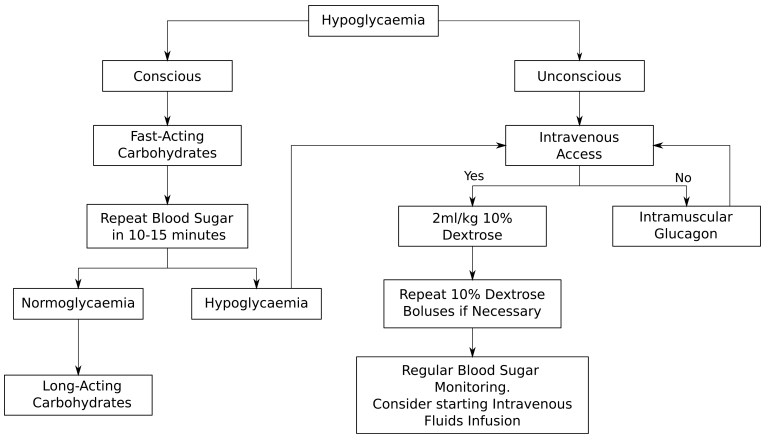

FIGURE 1
Emergency treatment of hypoglycaemia.

education alone is not sufficient to reduce adverse events from adrenal crises (5). A recent qualitative study of parents with young children with AI demonstrated that most of them followed the hydrocortisone oral stress dosing instructions prior to hospital admission, but only 2 out of 16 parents gave parenteral hydrocortisone at home, and the remaining patients received this from either the paramedics or emergency department staff (54). Psychological barriers to giving intramuscular hydrocortisone still remain a challenge for most carers and patients with AI. The hope for the future is an alternate form of parenteral hydrocortisone such as subcutaneous hydrocortisone injection becoming available for use in children with AI (55).

3.4 Future directions

Recent years have seen the development of modified-release hydrocortisone (MR-HC). Both the once-daily (56) and twice-daily (57) modified-released versions have been shown to be a closer mimic of the physiological circadian rhythm, compared to the conventional multiple-dose regimen. The modified-release preparation has a dual-release mechanism; an outer coating for an immediate release of hydrocortisone, and an inner core for a more sustained slower release. Studies have reported a significant reduction in body mass index, improved lipid profile, better glycaemic control and improved quality of life in those who are in the MR-HC group (58, 59). In a recent clinical trial comparing immediate-release and MR-HC in patients with CAH, both groups were able to achieve better 24-hour 17-OHP levels at 24 weeks compared to baseline, thus failing to prove their primary outcome. However, an extension of this clinical trial showed an overall improvement in disease control in the MR-HC group due to lower 17-OHP and androstenedione levels at multiple points throughout the day, with a significant reduction in 17-OHP variability. Dose reduction was also achievable in the MR-HC group whilst maintaining good disease control (60). In the United Kingdom, MR-HC has recently been approved for use in children and adolescents from 12 years of age with CAH. It will be exciting to see if this new modified-release therapy can reduce the incidence of hypoglycaemia in children with AI, especially during the early hours of the morning when they are most susceptible.

Identification of children with AI who experience recurrent nocturnal hypoglycaemia is also important to reduce occurrences. Continuous blood sugar monitoring (CGM) is currently mostly used in children with Type 1 diabetes, and less commonly used in those with Type 2 diabetes who are on insulin. Several studies conducted using CGM in adults with AI have shown benefits as they provided useful information on blood sugar trends. One study detected unrecognised nocturnal hypoglycaemia in 5 out of 6 adults with central hypoadrenalism causing early morning headaches. Full resolution of symptoms was achieved when the timings and dosages of the patients' hydrocortisone were altered based on the CGM readings (37). In a separate case study, a patient with Addison's disease reported better sleep when the evening dose of hydrocortisone was delayed due to detected hypoglycaemia from CGM (52). CGM in children with combined congenital central adrenal insufficiency and growth hormone deficiency have identified severe asymptomatic nocturnal hypoglycaemia (< 2.7mmol/L) in 3 out of 11 children, with the period of hypoglycaemia ranging from 30 to 150 minutes. On further review, they found that the daily doses of hydrocortisone were significantly lower in the 3 children and resolution of hypoglycaemia was seen when the doses were increased (49).

So why is CGM not widely used in hypoglycaemia disorders apart from diabetes? In a recent review by Worth et al. on the use of CGM, they found that hypoglycaemia sensitivity reduces as the threshold for hypoglycaemia decreases (61). The lowest hypoglycaemia sensitivity was reported at 17% with a hypoglycaemia threshold of 2.6mmol/L (62), as opposed to the highest hypoglycaemia sensitivity of only 86% with a hypoglycaemia threshold of 3.9mmol/L (63). The accuracy of CGM during a hypoglycaemia event is also variable and dependent on a lot of factors such as insulin levels and rapid glucose changes (64). Nevertheless, advancements in technology

and machine learning methods continue to improve and streamline hypoglycaemia predictive algorithms to improve accuracy. There is definitely a scope for the broader use of CGM, especially in children with recurrent hypoglycaemia. A recent large survey in the UK revealed a high demand for CGM in those patients with recurrent hypoglycaemia without diabetes and it was interesting to see how funding was obtained for some of these patients across the country. Parents and carers also described marked improvement in the quality of life with CGM use and a reduction in unplanned hospital admissions due to hypoglycaemia (65). In summary, the definitive role of CGM in the prevention of hypoglycaemia in children with AI is yet to be established and calls for larger multi-centre studies.

4 Conclusion

We have summarised the current literature on hypoglycaemia in AI and the significance of this problem as children are vulnerable to the effects of hypoglycaemia, especially young children with AI. We have discussed a few strategies to improve recognition of hypoglycaemia in individuals with AI including the use of CGM. However, the use of technology for blood sugar monitoring in children is currently only established in Type 1 diabetes. Future research is needed to utilise the advancement of technology in the management of AI and expand the role of CGM in children with AI. In the meanwhile, perhaps intermittent capillary or flash glucose monitoring in detecting those children with AI at risk of hypoglycaemia might be beneficial, with the hope that this will improve their neurodevelopmental outcome and reduce mortality rates from hypoglycaemia.

Author contributions

ZM had the presented idea. EB developed and outlined the abstract. SL performed the literature search and wrote the article. All authors contributed to the final manuscript, read and approved the submitted version.

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. Patti G, Guzzeti C, Di Iorgi N, Allegri AEM, Napoli F, Loche S, et al. Central adrenal insufficiency in children and adolescents. *Best Pract Res Clin Endocrinol Metab* (2018) 32:425–44. doi: 10.1016/j.beem.2018.03.012

2. Bornstein SR, Allolio B, Arlt W, Barthel A, Don-Wauchope A, Hammer GD, et al. Diagnosis and treatment of primary adrenal insufficiency: an endocrine society clinical practice guideline. *J Clin Endocrinol Metab* (2016) 101:364–89. doi: 10.1210/jc.2015-1710

3. Joersjo,¨ P, Block L. A challenging diagnosis that eventually results in a life-threatening condition: Addison's disease and adrenal crisis. *BMJ Case Rep CP* (2019) 12:e231858. doi: 10.1136/bcr-2019-231858

4. Hahner S, Loeffler M, Bleicken B, Drechsler C, Milovanovic D, Fassnacht M, et al. Epidemiology of adrenal crisis in chronic adrenal insufficiency: the need for new prevention strategies. *Eur J Endocrinol* (2010) 162:597. doi: 10.1530/EJE-09-0884

5. Rushworth RL, Torpy DJ, Stratakis CA, Falhammar H. Adrenal crises in children: perspectives and research directions. *Hormone Res Paediat* (2018) 89:341–51. doi: 10.1159/000481660

6. Park J, Didi M, Blair J. The diagnosis and treatment of adrenal insufficiency during childhood and adolescence. *Arch Dis Childhood* (2016) 101:860–5. doi: 10.1136/archdischild-2015-308799

7. Buchanan C, Preece M, Milner R. Mortality, neoplasia, and creutzfeldt-jakob disease in patients treated with human pituitary growth hormone in the United Kingdom. *Br Med J* (1991) 302:824–8. doi: 10.1136/bmj.302.6780.824

8. Taback S, Dean H. Mortality in canadian children with growth hormone (gh) deficiency receiving gh therapy 1967-1992. the canadian growth hormone advisory committee. *J Clin Endocrinol Metab* (1996) 81:1693–6. doi: 10.1210/jcem.81.5.8626817

9. Mills JL, Schonberger LB, Wysowski DK, Brown P, Durako SJ, Cox C, et al. Long-term mortality in the United States cohort of pituitary-derived growth hormone recipients. *J Pediatr* (2004) 144:430–6. doi: 10.1016/j.jpeds.2003.12.036

10. Sprague JE, Arbelaez,´ AM. Glucose counterregulatory responses to hypoglycemia. *Pediatr Endocrinol reviews: PER* (2011) 9:463.

11. De Feo P, Perriello G, Torlone E, Ventura MM, Fanelli C, Santeusanio F, et al. Contribution of cortisol to glucose counterregulation in humans. *Am J PhysiologyEndocrinol And Metab* (1989) 257:E35–42. doi: 10.1152/ajpendo.1989.257.1.E35

12. De Feo P, Perriello G, Torlone E, Ventura MM, Santeusanio F, Brunetti P, et al. Demonstration of a role for growth hormone in glucose counterregulation. *Am J Physiology-Endocrinol And Metab* (1989) 256:E835–43. doi: 10.1152/ajpendo.1989.256.6.E835

13. Tesfaye N, Seaquist ER. Neuroendocrine responses to hypoglycemia. *Ann New York Acad Sci* (2010) 1212:12–28. doi: 10.1111/j.1749-6632.2010.05820.x

14. Kim S-H, Park M-J. Effects of growth hormone on glucose metabolism and insulin resistance in human. *Ann Pediatr Endocrinol Metab* (2017) 22:145. doi: 10.6065/apem.2017.22.3.145

15. Kamba A, Daimon M, Murakami H, Otaka H, Matsuki K, Sato E, et al. Association between higher serum cortisol levels and decreased insulin secretion in a general population. *PloS One* (2016) 11:e0166077. doi: 10.1371/journal.pone.0166077

16. Wurzburger MI, Prelevic GM, Sonksen,¨ P, Balint-Peric LA, Wheeler M. The effect of recombinant human growth hormone on regulation of growth hormone secretion and blood glucose in insulin-dependent diabetes. *J Clin Endocrinol Metab* (1993) 77:267–72. doi: 10.1210/jcem.77.1.8325951

17. 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* (2021) 724. doi: 10.3389/fendo.2021.684011

18. Blasetti A, Chiuri RM, Tocco AM, Giulio CD, Mattei PA, Ballone E, et al. The effect of recurrent severe hypoglycemia on cognitive performance in children with type 1 diabetes: a meta-analysis. *J Child Neurol* (2011) 26:1383–91. doi: 10.1177/0883073811406730

19. Rizza RA, Mandarino LJ, Gerich JE. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action. *J Clin Endocrinol Metab* (1982) 54:131–8. doi: 10.1210/jcem-54-1-131

20. Nosadini R, Del Prato S, Tiengo A, Valerio A, Muggeo M, Opocher G, et al. Insulin resistance in cushing's syndrome. *J Clin Endocrinol Metab* (1983) 57:529–36. doi: 10.1210/jcem-57-3-529

21. Divertie GD, Jensen MD, Miles JM. Stimulation of lipolysis in humans by physiological hypercortisolemia. *Diabetes* (1991) 40:1228–32. doi: 10.2337/diab.40.10.1228

22. Samra JS, Clark ML, Humphreys SM, MacDonald IA, Bannister PA, Frayn KN. Effects of physiological hypercortisolemia on the regulation of lipolysis in subcutaneous adipose tissue. *J Clin Endocrinol Metab* (1998) 83:626–31. doi: 10.1210/jc.83.2.626

23. Christiansen JJ, Djurhuus CB, Gravholt CH, Iversen P, Christiansen JS, Schmitz O, et al. Effects of cortisol on carbohydrate, lipid, and protein metabolism: studies of acute cortisol withdrawal in adrenocortical failure. *J Clin Endocrinol Metab* (2007) 92:3553–9. doi: 10.1210/jc.2007-0445

24. Weise M, Drinkard B, Mehlinger SL, Holzer SM, Eisenhofer G, Charmandari E, et al. Stress dose of hydrocortisone is not beneficial in patients with classic congenital adrenal hyperplasia undergoing short-term, high-intensity exercise. *J Clin Endocrinol Metab* (2004) 89:3679–84. doi: 10.1210/jc.2003-032051

25. Wurtman R, Pohorecky L. Adrenocortical control of epinephrine synthesis in health and disease. *Adv Metab Disord* (1971) 5:53–76. doi: 10.1016/B978-0-12-027305-8.50022-9

26. Weise M, Mehlinger SL, Drinkard B, Rawson E, Charmandari E, Hiroi M, et al. Patients with classic congenital adrenal hyperplasia have decreased epinephrine reserve and defective glucose elevation in response to high-intensity exercise. *J Clin Endocrinol Metab* (2004) 89:591–7. doi: 10.1210/jc.2003-030634

27. Merke DP, Chrousos GP, Eisenhofer G, Weise M, Keil MF, Rogol AD, et al. Adrenomedullary dysplasia and hypofunction in patients with classic 21-hydroxylase deficiency. *New Engl J Med* (2000) 343:1362–8. doi: 10.1056/NEJM200011093431903

28. DeRosa MA, Cryer PE. Hypoglycemia and the sympathoadrenal system: neurogenic symptoms are largely the result of sympathetic neural, rather than adrenomedullary, activation*Am J Physiol-Endocrinol Metab* (2004) 287:E32–41 doi: 10.1152/ajpendo.00539.2003

29. Morita S, Otsuki M, Izumi M, Asanuma N, Izumoto S, Saitoh Y, et al. Reduced epinephrine reserve in response to insulin-induced hypoglycemia in patients with pituitary adenoma. *Eur J Endocrinol* (2007) 157:265–70. doi: 10.1530/EJE-07-0176

30. Macdessi JS, Randell TL, Donaghue KC, Ambler GR, van Asperen PP, Mellis CM. Adrenal crises in children treated with high-dose inhaled corticosteroids for asthma. *Med J Aust* (2003) 178:214–6. doi: 10.5694/j.1326-5377.2003.tb05165.x

31. Hussain K, Hindmarsh P, Aynsley-Green A. Neonates with symptomatic hyperinsulinemic hypoglycemia generate inappropriately low serum cortisol counterregulatory hormonal responses. *J Clin Endocrinol Metab* (2003) 88:4342–7. doi: 10.1210/jc.2003-030135

32. Hahner S, Spinnler C, Fassnacht M, Burger-Stritt S, Lang K, Milovanovic D, et al. High incidence of adrenal crisis in educated patients with chronic adrenal insufficiency: a prospective study. *J Clin Endocrinol Metab* (2015) 100:407–16. doi: 10.1210/jc.2014-3191

33. Smans LC, van der Valk ES, Hermus AR, Zelissen PM. Incidence of adrenal crisis in patients with adrenal insufficiency. *Clin Endocrinol* (2016) 84:17–22. doi: 10.1111/cen.12865

34. Reisch N, Willige M, Kohn D, Schwarz H-P, Allolio B, Reincke M, et al. Frequency and causes of adrenal crises over lifetime in patients with 21-hydroxylase deficiency. *Eur J Endocrinol* (2012) 167:35. doi: 10.1530/EJE-12-0161

35. Odenwald B, Nennstiel-Ratzel U, Doerr H-G, Schmidt H, Wildner M, Bonfig W. Children with classic congenital adrenal hyperplasia experience salt loss and hypoglycemia: evaluation of adrenal crises during the first 6 years of life. *Eur J Endocrinol* (2016) 174:177–86. doi: 10.1530/EJE-15-0775

36. Kawahara T, Tsuji M, Tominaga N, Toyama N, Toda M. Frequency of adrenal insufficiency in patients with hypoglycemia in an emergency department: A cross-sectional study. *J Endocrine Soc* (2022) 6:bvac119. doi: 10.1210/jendso/bvac119

37. Watanabe T, Ozawa A, Ishii S, Tomaru T, Shibusawa N, Saito T, et al. Usage of continuous glucose monitoring (cgm) for detecting an unrecognized hypoglycemia and management of glucocorticoid replacement therapy in adult patients with central hypoadrenalism. *Endocrine J* (2018) 65(5):547–56. doi: 10.1507/endocrj.EJ16-0387

38. Artavia-Loria E, Chaussain J, Bougneres P, Job J. Frequency of hypoglycemia in children with adrenal insufficiency. *Eur J Endocrinol* (1986) 113:S275–8. doi: 10.1530/acta.0.112S275

39. Donaldson M, Thomas PH, Love JG, Murray G, McNinch AW, Savage D. Presentation, acute illness, and learning difficulties in salt wasting 21-hydroxylase deficiency. *Arch Dis childhood* (1994) 70:214–8. doi: 10.1136/adc.70.3.214

40. Pinto G, Tardy V, Trivin C, Thalassinos C, Lortat-Jacob S, Nihoul-Fekete C, et al. Followup of 68 children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency: relevance of genotype for management. *J Clin Endocrinol Metab* (2003) 88:2624–33. doi: 10.1210/jc.2002-021433

41. Keil MF, Bosmans C, Van Ryzin C, Merke DP. Hypoglycemia during acute illness in children with classic congenital adrenal hyperplasia. *J Pediatr Nurs* (2010) 25:18–24. doi: 10.1016/j.pedn.2008.06.003

42. Laghari TM, Ibrahim MN, Khoso Z, Hanif MI, Meher-Un N, Raza J. Spectrum of addison's disease in children. *J Coll Physicians Surg Pak* (2020) 30:1086–9. doi: 10.29271/jcpsp.2020.10.1086

43. Ngaosuwan K, Johnston DG, Godsland IF, Cox J, Majeed A, Quint JK, et al. Increased mortality risk in patients with primary and secondary adrenal insufficiency. *J Clin Endocrinol Metab* (2021) 106:e2759–68. doi: 10.1210/clinem/dgab096

44. Grosse SD, Van Vliet G. How many deaths can be prevented by newborn screening for congenital adrenal hyperplasia? *Hormone Res Paediatrics* (2007) 67:284–91. doi: 10.1159/000098400

45. Swerdlow AJ, Higgins CD, Brook CG, Dunger DB, Hindmarsh PC, Price DA, et al. Mortality in patients with congenital adrenal hyperplasia: a cohort study. *J Pediatr* (1998) 133:516–20. doi: 10.1016/S0022-3476(98)70060-5

46. Kovacs,´ J, Votava F, Heinze G, Solyom,´ J, Lebl J, Pribilincova,´ Z, et al. Lessons from 30 years of clinical diagnosis and treatment of congenital adrenal hyperplasia in five middle european countries. *J Clin Endocrinol Metab* (2001) 86:2958–64. doi: 10.1210/jc.86.7.2958

47. Falhammar H, Frisen,´ L, Norrby C, Hirschberg AL, Almqvist C, Nordenskjold,¨ A, et al. Increased mortality in patients with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* (2014) 99:E2715–21. doi: 10.1210/jc.2014-2957

48. Vermes I, Dohanics J, Toth G, Pongracz J. Maturation of the circadian rhythm of the adrenocortical functions in human neonates and infants. *Hormone Res Paediatrics* (1980) 12:237–44. doi: 10.1159/000179126

49. 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* (2013) 79:232–7. doi: 10.1111/cen.12123

50. Mazziotti G, Formenti A, Frara S, Roca E, Mortini P, Berruti A, et al. Management of endocrine disease: risk of overtreatment in patients with adrenal insufficiency: current and emerging aspects. *Eur J Endocrinol* (2017) 177:R231–48. doi: 10.1530/EJE-17-0154

51. Neumann U, Braune K, Whitaker MJ, Wiegand S, Krude H, Porter J, et al. A prospective study of children aged 0–8 years with cah and adrenal insufficiency treated with hydrocortisone granules. *J Clin Endocrinol Metab* (2021) 106:e1433–40. doi: 10.1210/clinem/dgaa626

52. Meyer G, Hackemann A, Reusch J, Badenhoop K. Nocturnal hypoglycemia identified by a continuous glucose monitoring system in patients with primary adrenal insufficiency (addison's disease). *Diabetes Technol Ther* (2012) 14:386–8. doi: 10.1089/dia.2011.0158

53. Johnstone HC, McNally RJ, Cheetham TD. The impact of fasting and treatment omission on susceptibility to hypoglycaemia in children and adolescents with gh and cortisol insufficiency. *Clin Endocrinol* (2008) 69:436–42. doi: 10.1111/j.1365-2265.2008.03210.x

54. Worth C, Vyas A, Banerjee I, Lin W, Jones J, Stokes H, et al. Acute illness and death in children with adrenal insufficiency. *Front Endocrinol* (2021) 1264. doi: 10.3389/fendo.2021.757566

55. Hahner S, Burger-Stritt S, Allolio B. Subcutaneous hydrocortisone administration for emergency use in adrenal insufficiency. *Eur J Endocrinol* (2013) 169:147–54. doi: 10.1530/EJE-12-1057

56. Johannsson G, Nilsson A, Bergthorsdottir R, Burman P, Dahlqvist P, Ekman B, et al. Improved cortisol exposure-time profile and outcome in patients with adrenal insufficiency: a prospective randomized trial of a novel hydrocortisone dual-release formulation. *J Clin Endocrinol Metab* (2012) 97:473–81. doi: 10.1210/jc.2011-1926

57. Mallappa A, Sinaii N, Kumar P, Whitaker MJ, Daley L-A, Digweed D, et al. A phase 2 study of chronocort, a modified-release formulation of hydrocortisone, in the treatment of adults with classic congenital adrenal hyperplasia. *J Clin Endocrinol Metab* (2015) 100:1137–45. doi: 10.1210/jc.2014-3809

58. Bannon CA, Gallacher D, Hanson P, Randeva HS, Weickert MO, Barber TM. Systematic review and meta-analysis of the metabolic effects of modified-release hydrocortisone versus standard glucocorticoid replacement therapy in adults with adrenal insufficiency. *Clin Endocrinol* (2020) 93:637–51. doi: 10.1111/cen.14275

59. Delle Cese F, Corsello A, Cintoni M, Locantore P, Pontecorvi A, Corsello SM, et al. Switching from immediate-release to fractionated dual-release hydrocortisone may improve metabolic control and qol in selected primary adrenal insufficiency patients. *Front Endocrinol* (2021) 11:610904. doi: 10.3389/fendo.2020.610904

60. Merke DP, Mallappa A, Arlt W, Brac de la Perriere A, Linden´ Hirschberg A, Juul A, et al. Modified-release hydrocortisone in congenital adrenal hyperplasia. *J Clin Endocrinol Metab* (2021) 106:e2063–77. doi: 10.1210/clinem/dgab051

61. Worth C, Hoskyns L, Salomon-Estebanez M, Nutter PW, Harper S, Derks TGJ, et al. Continuous glucose monitoring for children with hypoglycaemia: evidence in 2023. *Front Endocrinol* (2023) 14:1116864. doi: 10.3389/fendo.2023.1116864

62. Beardsall K, Vanhaesebrouck S, Ogilvy-Stuart A, Vanhole C, Midgley P, Thio M, et al. Validation of the continuous glucose monitoring sensor in preterm infants. *Arch Dis Childhood-Fetal Neonatal Edition* (2013) 98:F136–40. doi: 10.1136/archdischild-2012-301661

63. Rayannavar A, Elci OU, Mitteer L, De Leon,´ DD. Continuous glucose monitoring systems: are they useful for evaluating glycemic control in children with hyperinsulinism? *Hormone Res paediat* (2019) 92:319–27. doi: 10.1159/000506230

64. 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

65. Ng SM, Dearman S, Fisher M, Mushtaq T, Randell T. Paediatric society and hyperinsulinism charity national surveys on cgm access for patients with recurrent hypoglycaemia. *J Endocrine Soc* (2023) 7:bvad021. doi: 10.1210/jendso/bvad021

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Brain magnetic resonance imaging review suggests unrecognised hypoglycaemia in childhood

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Introduction: Neonatal and early-life hypoglycaemia, is a frequent finding but is often non-specific and asymptomatic, making detection and diagnosis challenging. Hypoglycaemia-induced cerebral injury can be identified by magnetic resonance imaging (MRI) changes in cerebral white matter, occipital lobes, and posterior parietotemporal regions. It is unknown if children may have hypoglycaemic brain injury secondary to unrecognised hypoglycaemia in early life. We have examined retrospective radiological findings of likely brain injury by neuroimaging to investigate the existence of previous missed hypoglycaemic events.

Methods: Retrospective MRI data in children in a single tertiary centre, over a ten-year period was reviewed to identify potential cases of unrecognised early-life hypoglycaemia. A detailed search from an electronic radiology repository involved the term "hypoglycaemia'' from text-based reports. The initial report was used for those who required serial scanning. Images specific to relevant reports were further reviewed by a designated paediatric neuroradiologist to confirm likely hypoglycaemia induced brain injury. Medical records of those children were subsequently reviewed to assess if the hypoglycaemia had been diagnosed prior to imaging.

Results: A total of 107 MR imaging reports were identified for review, and 52 (48.5%) showed typical features strongly suggestive of hypoglycaemic brain injury. Medical note review confirmed no documented clinical information of hypoglycaemia prior to imaging in 22 (42%) patients, raising the likelihood of missed hypoglycaemic events resulting in brain injury.

Conclusions: We have identified the existence of unrecognised childhood hypoglycaemia through neuroimaging review. This study highlights the need for heightened awareness of early life hypoglycaemia to prevent adverse neurological outcomes later in childhood.

KEYWORDS

hypoglycaemia, child, magnetic resonance imaging, brain injury, glucose

Introduction

Neonatal hypoglycaemia is a common occurrence but exists along a wide spectrum of severity. There is evidence that untreated, or inadequately treated, early life hypoglycaemia may cause later life neurological injury (1). This is particularly prominent when both glucose and alternative brain fuel sources are simultaneously suppressed, such as in patients with congenital hyperinsulinism (2), irrespective of the permanence of disease (3).

The screening process for neonatal hypoglycaemia is subject to significant variation (4). This variation is partly dependent on the adoption of certain criteria for blood glucose testing, as well as differing hypoglycaemia thresholds for further investigation and treatment (5). As blood glucose testing is not universal, and consensus on what constitutes a "normal blood glucose" is not clear, there is a possibility for missed hypoglycaemia, with or without hyperinsulinism (6). Any missed hypoglycaemia has the potential to cause brain injury and result in neurological manifestations such as seizures and developmental delay (7). Clinicians and families living with Congenital Hyperinsulinism have been concerned that current newborn screening methods are not wholly adequate, resulting in missed neonatal hypoglycaemia presenting later as neurodisability (6). Therefore, it is important to examine this missed evidence through retrospective review, including an assessment of brain scans to detect imprints from prior neuroglycopaenia.

Brain injury secondary to hypoglycaemia preferentially affects the parietal and occipital regions of the brain and can be appreciated on magnetic resonance imaging (MRI) scans (8, 9) with high specificity and positive predictive value. MRI changes in these anatomical areas are strongly suggestive of hypoglycaemic injury and do not suggest alternative aetiologies with a high degree of certainty (9, 10). The existence of radiological evidence of brain impact from previously unrecognised hypoglycaemia has not been evaluated and could provide insight into the possibility of missed and detrimental early-life hypoglycaemia. We have undertaken neuroimaging retrospective review to identify such potential radiological evidence for hypoglycaemia.

Aim

To evaluate for the existence of any case of missed early-life hypoglycaemia as detected radiologically by magnetic resonance neuroimaging.

Methods

To detect the possibility of brain changes from prior neuroglycopaenia, we undertook a retrospective review of brain MRI reports over a 10-year period (Dec 2011 to Dec 2021) for a quality improvement project investigating imaging outcomes at a single tertiary referral centre (Royal Manchester Children's Hospital). As the aim was to detect the existence of any (minimum one) case of missed hypoglycaemia, the study design was retrospective, and a quality improvement method was used. The study received approval from the Children's Clinical Audit Committee at the Royal Manchester Children's Hospital (registration 13 Feb 2023). The focus of the study was not to derive a comprehensive list of patients with radiological evidence of early-life hypoglycaemia; instead, retrospective sampling over a 10-year period was adopted as a pragmatic method to interrogate a large database to test the hypothesis that there might be patients with radiological evidence of early-life hypoglycaemia that had not been clinically detected (missed hypoglycaemia).

To this effect, an electronic neuroimaging database in Picture Archiving and Communication System (PACS®) at the Royal Manchester Children's Hospital site was reviewed for the search term "hypoglycaemia" in radiology text reports. The search was undertaken by one operator using manufacturer-specified electronic strategy without the need for additional codes for data extraction or the development of a standard operating procedure or manual. A wider search strategy to include "occipital or parietal lobe changes" was not adopted. This was to limit the generation of false positives requiring timely and costly verification.

The search field included all children (under 16 years at the time of data extraction) referred for an MRI scan, regardless of the reason specified in the requesting information. Text reports were obtained from the initial scan if multiple, serial scans were undertaken, and scans included if the findings indicated hypoglycaemic injury. T2-weighted, coronal fluid-attenuated inversion recovery [FLAIR]-weighted imaging MR sequences were used to identify radiological characteristics of hypoglycaemia in occipital and parietal lobes (10, 11). Neuroimaging findings typical of hypoglycaemia were further reviewed by a paediatric neuro-radiologist who re-analysed images to confirm or refute findings of likely hypoglycaemic brain injury. Patients with brain changes not typical of hypoglycaemia were excluded from investigation. As this study examined text reports retrospectively, text varied in style and syntax, and outcome was binary (hypoglycaemia evidence yes/no), it was not possible to test inter-rater reliability. However, the study team neuro-radiologist was designated to review all scans with positive evidence of hypoglycaemia, ensuring cross checking of scan reporting accuracy to reduce false positives and increase the specificity of findings.

All patients identified by text searching were born in St Mary's Hospital and Managed Clinical Services and adjoining maternity hospitals in the Manchester region where the regional neonatal network guideline based on the British Association for Perinatal Medicine neonatal hypoglycaemia framework was utilised. Therefore, it was assumed that all children with radiological evidence of hypoglycaemia had been subject to a common newborn hypoglycaemia screening process. However, specific perinatal data including glucose measurements were not obtained for all patients.

The patient names of those with confirmed likely hypoglycaemic injury on MRI were then matched with those in hospital electronic health records and an additional database of patients with congenital hyperinsulinism maintained by the Northern Congenital Hyperinsulinism (NORCHI) service manually by one member of the study team. Patients with prior

recorded information of hypoglycaemia and/or congenital hyperinsulinism as diagnosis were filtered out as exclusion criteria. Thus, patients with previously documented hypoglycaemia, or those in whom hypoglycemia was considered a possibility by the referring clinician prior to imaging, were excluded. For children born outside the maternity hospital linked to our centre, maternity and neonatal information was not obtained. Patients with no documented history of hypoglycaemia, and those in whom hypoglycaemia was not suggested or suspected on medical review prior to imaging, were included for review. As per quality improvement methodology, parents or carers were not contacted for further information to confirm absence of hypoglycaemia. For study purposes, only text-based clinician interpretation was used to identify early-life hypoglycaemia. A numerical definition of hypoglycaemia was not used, as this would be less relevant in the context of patients without documented hypoglycaemia in the absence of a widely accepted consensus on specific cutoffs.

For a quality improvement study design with focus on the determination of radiological evidence of hypoglycaemia in patients without documented hypoglycaemia, extensive data capture (such as a wide range of demographic variables) was limited to a need-to-know basis in keeping with principles of good governance. This ensured unnecessary data usage beyond the remit of the study design. This study was not designed to test hypoglycaemia severity with the extent of radiological injury as reported elsewhere (12); therefore, patients with information on hypoglycaemia were excluded for analysis of their reports.

Results

The initial search for "hypoglycaemia" identified 107 MRI results, of which 52 (48.5%) reported evidence of hypoglycaemic brain injury (Figure 1). Seizures, developmental delay and learning difficulties were the most common indications for MR imaging referral. All reported hypoglycaemic brain injury images were confirmed on reanalysis.

At the time of imaging, patients varied in age with a median (range) age of 0.24 (0 –9) years. Review of health records and imaging requests revealed a history of definite or likely hypoglycaemia in 26 (50%) patients. No clinical details were accessible in four patients. In the remaining 22 patients (42% of all scans characteristic of hypoglycaemia) in whom clinical details at referral were available, there was no evidence of: a clinical diagnosis which could include hypoglycaemia; clinical suspicion of hypoglycaemia or; treatment for hypoglycaemia. As individual patients were not contacted, the absence of hypoglycaemia was not confirmed with families. However, these findings strongly suggest that early life hypoglycaemia is likely to have been unrecognised in at least 22 patients over a 10-year period (Figure 1).

Patient characteristics, with later life clinical presentation and MRI scan findings have been provided in Table 1. Within the missed hypoglycaemia group, 15 (68%) were female and median (range) age was 2.08 (0-14) years. At the time of referral, the following phenotypes were noted – seizures (6), learning difficulties (5), developmental delay (5), paresis (2), hypertonia (1), recurrent apnoea (1), tip toe gait (1) and meningitis (1) (Figure 2). All patients had uncomplicated births with no evidence of perinatal stress to suggest possible transient hyperinsulinism. Two patients were born prematurely between 30 and 35 weeks of gestation but no hypoglycaemia was identified during the neonatal period. All patients with imaging findings of hypoglycaemic brain injury showed either occipital (13) or parieto-occipital (9) changes in white matter in both hemispheres on T2 scanning, typical of early-life hypoglycaemia.

Discussion

Our retrospective review of patients with radiological evidence of brain injury strongly suggests that a proportion sustained their brain damage due to unrecognised, early-life hypoglycaemia. Our findings support concerns of missed hypoglycaemia raised by patient organisations such as Congenital Hyperinsulinism International (6) and align with recent observations correlating

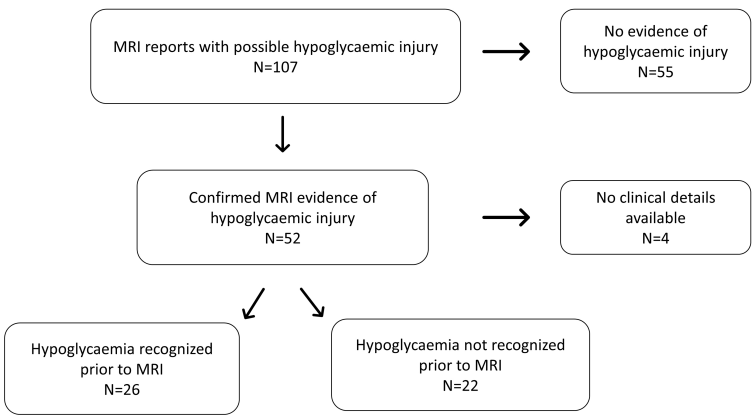

FIGURE 1
Flowchart diagram illustrating the distribution of patients included in the study (MRI-Magnetic Resonance Imaging).

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TABLE 1 Clinical findings and MRI report from all patients considered to have possible missed hypoglycaemia.

	Clinical presentation	MRI findings
1.	Right-sided unprovoked seizures with secondary generalised seizures	Bilateral occipital gyral volume loss, more so on the right.
2.	Tip toe walking. Ex-preterm, severe intra-uterine growth restriction (IUGR)	There is abnormality in the left occipital lobe with what appear to be a focal area of gliosis and cortical atrophy, Periventricular high signal is noted in the occipital lobe on the right raising the possibility of localised gliosis.
3.	Developmental delay, dyspraxia	Bilateral symmetrical abnormal high intensity signal seen in the deep white matter of both occipital lobes with prominence in perivascular spaces.
4.	Increasing Head Circumference, Learning difficulties	T2/FLAIR high signal in the right occipital white matter extending from the periventricular deep white matter to the subcortical white matter, consistent with gliosis.
5.	Nystagmus, Learning difficulties	Gyral crowding involving occipital lobes worse on the left than the right with some prominence to the overlying CSF space.
6.	Developmental delay	Mild asymmetry of the ventricular system, with prominent right occipital horn and right occipital sulcal prominence compared to the left, presumably ex-vacuo dilatation of the right occipital horn due to ipsilateral occipital parenchymal volume loss.
7.	Learning difficulties	Bilateral cystic encephalomalacia/gliosis in the fronto-temporoparietal-occipital lobe.
8.	Developmental delay	Features suggestive of occipital lobe atrophy.
9.	Hypertonia in Lower Limb	Periventricular Leukomalacia with impaired myelination extending into the deep fronto-parietal white matter, prematurity related. Bilateral cortico-subcortical signal abnormality in the parieto-occipital lobes associated with volume loss.
10.	Microcephaly, Learning difficulties	Medial occipital lobe volume loss and high signal change within the white matter.
11	Weakness in right arm	Generalised prominence of extra-axial CSF over the left occipital lobe raising the possibility of some underlying atrophy.
12.	Microcephaly, drug resistant epilepsy	Mild prominence of cerebral and cerebellar sulci, more prominent in the parieto-occipital lobes bilaterally.
13.	Lethargy, floppy	Bilateral parieto-occipital volume loss + bilateral abnormality in the globi pallidi in association with mild cerebellar atrophy.
14.	Developmental delay, neonatal abstinence syndrome	Evidence of white matter loss and gyral crowding in both occipital lobes.
15.	Generalised seizure	High signal on the diffusion sequences in the parieto-occipital region.
16.	Recurrent apnoea	Bilateral symmetrical T2 high signal in the subcortical white matter in the parietal occipital lobes with diffusion changes and loss of cortical ribbon in same areas.
17.	Reduced GCS, meningitis	Bilateral occipital changes (Rt > Lt).
18.	Weakness and right-sided, severe delay in speech and language	Significant bilateral occipital lobe changes.
19.	Epilepsy, recent generalised tonic-clonic seizures	Bilateral medial occipital lobe subcortical white matter hyperintense change. Volume sequence shows some cortical volume loss on the right.
20	increasing sacral dimple	Encephalomalacia/gliosis with volume loss in the left parasagittal parieto-occipital lobes with cortical involvement.
21.	Hypoxic ischaemic encephalopathy, seizures	Bilateral slight increase T2 high signal in the parietal occipital white matter.
22	Poorly controlled epilepsy, learning difficulties	Bilateral parietal occipital abnormalities; also has left frontal lobe abnormality.

MRI findings with a history of hypoglycaemia (8). Our findings also support hypoglycaemia as potential aetiology for unexplained neurodevelopmental abnormalities presenting in later life.

Our study provides initial but important evidence for a preventable cause of long-term neurodisability, adding support to calls for greater monitoring for the risk of hypoglycaemia in early life, particularly in the neonatal period. However, we accept that our study was retrospective, restricted to a single centre and that causal association, chiefly that with congenital hyperinsulinism, was not explored comprehensively. This study was further limited by incomplete information in a few cases, with potential for improved diagnostic pointers about the nature of hypoglycaemia. Nonetheless, absent information in a minority of patients does not digress from the importance of the main finding, that of missed and unrecognised hypoglycaemia leading to brain injury.

Our study was not focused on the comprehensive evaluation of the prevalence of hypoglycaemia related brain injury in early life or the association of the degree and duration of glycaemic excursion from normal for each child, as demonstrated in other studies (12). Instead, our study successfully focused on potential missed opportunities to detect early life hypoglycaemia.

Our study used typical scan findings based on occipital and parietal (or both) lobe changes to identify radiological evidence of hypoglycaemia. MR scan based radiological evidence is specific to early-life hypoglycaemia (12) with 82% positive predictive value in a large case series (9), suggesting that scan findings in our cohort did indeed reflect true evidence for hypoglycaemic brain injury. Other differentials of these MRI findings are possible but, given the high

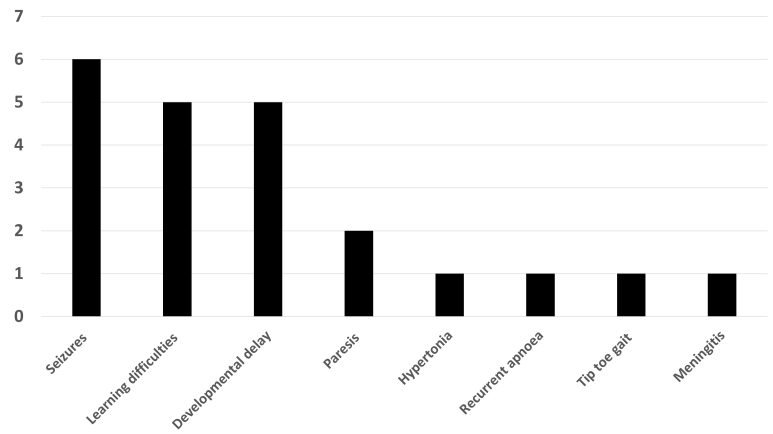

FIGURE 2
Bar chart showing the distribution of clinical findings in patients with neuroimaging findings suggestive of hypoglycaemic injury.

predictive value for hypoglycaemia, not worth visiting in depth. We did not use deep grey matter loss (for instance in the caudate nucleus) as another measure of hypoglycaemia (13) as our priority was specificity over sensitivity. While deep grey matter loss has been described, significant volume loss criteria have not been developed for use as a clinical tool. It is possible that future studies may identify grey matter parameters correlating with the extent and severity of neuroglycopaenia related brain injury.

While our study results provide insight into the difficulty of recognition of clinically important hypoglycaemia in early life, they do not provide information about safe levels of glucose in infancy, or those who should be considered for screening of hypoglycaemia. As our study design only incorporated the assessment of the initial scan report, disregarding later serial scan reports, radiological improvement was not documented. However, the absence of a longitudinal element in our study is not a fundamental flaw, as the successful cross-sectional determination of radiological evidence of hypoglycaemia was sufficient to address the study's aim. Our study findings may be used as a steppingstone to design large multi-centric studies testing severity and duration of hypoglycaemia to ascertain the true prevalence of hypoglycaemia mediated brain injury. Nonetheless, the recognition of the existence of missed early-life hypoglycaemia, with potential for deleterious neurodevelopmental consequences, prompts the need for further development of neonatal hypoglycaemia screening practices. Additionally, our study findings point to the consideration for missed hypoglycaemia in diagnostic checklists for clinicians ordering MRI scans for neurodevelopmental abnormalities presenting in later life.

Although our study was not designed to address the true prevalence of missed opportunities for neonatal identification of hypoglycaemia, we have identified a significant number of hypoglycaemia related brain changes suggestive of early-life unrecognised hypoglycaemia. It is possible that our study missed other cases of hypoglycaemia; for this, a larger study involving multiple centres will be required. Such studies may require longitudinal follow-up to correlate the severity of neonatal hypoglycaemia and identify contributory aggravating factors to provide a fuller description informing causality. This study points to the need for greater awareness and the need to refocus on diagnostic frameworks for neonatal hypoglycaemia.

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

CW: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. PG: Investigation, Writing – review & editing. KR: Investigation, Writing – review & editing. SW: Data curation, Writing – review & editing. MS: Writing – review & editing. AM: Data curation, Supervision, Writing – review & editing. IB: Methodology, Supervision, Writing – review & editing.

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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.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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References

1. Lucas A, Morley R, Cole TJ. Adverse neurodevelopmental outcome of moderate neonatal hypoglycaemia. *Br Med J*. (1988) 297:1304–8. doi: 10.1136/bmj.297.6659.1304

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

3. 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

4. Thompson-Branch A, Havranek T. Neonatal hypoglycemia. *Pediatr Rev*. (2017) 38:147–57. doi: 10.1542/pir.2016-0063

5. Shaikh G, Lucas-Herald A, Dastamani A, Salomon-Estebanez M, Senniappan S, Abid N, et al. Standardised practices in the networked management of congenital hyperinsulinism: a UK national collaborative consensus. *Front Endocrinol (Lausanne)*. (2023) 14:1231043. doi: 10.3389/fendo.2023.1231043

6. 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 parents and families. *Orphanet J Rare Dis*. (2022) 17:61. doi: 10.1186/s13023-022-02214-y

7. Tam EWY, Haeusslein LA, Bonifacio SL, Glass HC, Rogers EE, Jeremy RJ, et al. Hypoglycemia is associated with increased risk for brain injury and adverse neurodevelopmental outcome in neonates at risk for encephalopathy. *J Pediatr*. (2012) 161:88–93. doi: 10.1016/j.jpeds.2011.12.047

8. Uyur Yalçin E, Maraş Genç H, Bayhan A, Anık Y, Kara B. Neurodevelopmental outcome in patients with typical imaging features of injury as a result of neonatal hypoglycemia. *Noropsikiyatri Ars*. (2022) 59:296–302. doi: 10.29399/npa.27997

9. Wong DST, Poskitt KJ, Chau V, Miller SP, Roland E, Hill A, et al. Brain injury patterns in hypoglycemia in neonatal encephalopathy. *Am J Neuroradiol*. (2013) 34:1456–61. doi: 10.3174/ajnr.A3423

10. Barkovich AJ, Ali FA, Rowley HA, Bass N. Imaging patterns of neonatal hypoglycemia. *Am J Neuroradiol*. (1998) 19:523–8.

11. Burns CM, Rutherford MA, Boardman JP, Cowan FM. Patterns of cerebral injury and neurodevelopmental outcomes after symptomatic neonatal hypoglycemia. *Pediatrics*. (2008) 122:65–74. doi: 10.1542/peds.2007-2822

12. Gu M-H, Amanda F, Yuan T-M. Brain injury in neonatal hypoglycemia: A hospital-based cohort study. *Clin Med Insights: Pediatrics*. (2019) 13. doi: 10.1177/1179556519867953

13. Nivins S, Kennedy E, McKinlay C, Thompson B, Harding JE. Children with Hypoglycemia and Their Later Development (CHYLD) Study Team. Size at birth predicts later brain volumes. *Sci Rep*. (2023) 13:12446. doi: 10.1038/s41598-023-39663-9

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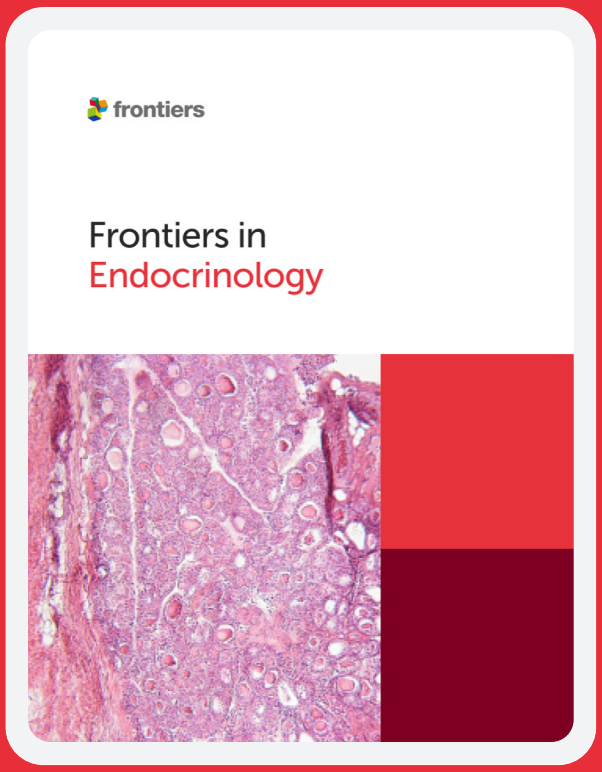

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