ENDOCRINE DISEASES OF NEWBORN: EPIDEMIOLOGY, PATHOGENESIS, THERAPEUTIC OPTION AND OUTCOME

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ENDOCRINE DISEASES OF NEWBORN: EPIDEMIOLOGY, PATHOGENESIS, THERAPEUTIC OPTION AND OUTCOME

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EDITORIAL
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Editorial: Endocrine Diseases of Newborn: Epidemiology, Pathogenesis, Therapeutic Options and Outcome

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Keywords: glucose homeostasis, calcium homeostasis, hypopituitarism, adrenal insufficiency, differences in sex development, minipuberty, congenital adrenal hyperplasia, neonatal bone disorders

Editorial on the Research Topic

Endocrine Diseases of Newborn: Epidemiology, Pathogenesis, Therapeutic Option and Outcome

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The aim of this collection is to provide information on recent advances and current thinking in selected areas of Neonatal Endocrinology, as we believe that this area can be challenging in presentation and management for both neonatologists and pediatric endocrinologists. To this end, we invited several leaders in the field to contribute. In addition, we have included 3 manuscripts submitted to Frontiers that fell within the remit of our topic.

The collection focuses on selected conditions presenting in the newborn consisting of a series of 10 mini-reviews, including some which may be considered the "go to" article for up-to-date science and management of various conditions.

The neonatal period is complicated by unique physiology, because of the transition from intrauterine life including the influence of maternal and placental hormones, and "normal" values for hormone levels can be difficult to define, and many change with increasing postnatal age. The measurement of hormone levels is further complicated by numerous additional circulating steroids which may interfere with assays, and the challenges of small sample size. In the context of this milieu the collection of articles ranges from common neonatal problems such as hypoglycemia to rare disorders such as neonatal bone disease. The content of all the articles in the collection is summarized below.

Bosch i Ara et al. provide an extensive review of congenital hypopituitarism, with a thorough review of the science, including the known genetics, phenotype-genotype correlations, syndromic, and non-syndromic hypopituitarism with helpful accompanying tables. They also provide a comprehensive guide to the clinical presentation including red-flag symptoms and signs, diagnosis, assessment and management of pituitary hormone deficiencies.

Di Dalmazi et al. present a study on the effect of maternal and neonatal factors on neonatal TSH levels, using retrospective data from screening for congenital hypothyroidism in 62,132 infants in

Abruzzo, Italy, a relatively iodine deficient area. As both sex and postnatal age at collection affected the TSH level, they advocate the use of locally derived TSH cut-offs for both and provide local percentile charts for TSH based on their data. Effects of maternal and neonatal factors were modest, but the study was limited by data routinely collected in the screening programme and the unknown contribution that iodine deficiency may have played in this population.

Buonocore et al. describe causes of adrenal insufficiency, with a focus on genetic conditions that present in the first few months of life. This superb overview provides clear and concise information on complex pathways and conditions, identifying seminal features of presentation in a manner that is easy to read. Testing for adrenal insufficiency was not in the remit of this invited review, but assessment of the HPA axis can be found in the review of congenital hypopituitarism by Bosch i Ara et al..

Balsamo et al. explore the breadth of conditions presenting as congenital adrenal hyperplasia in the newborn period, and provide insight into some of the rarer conditions. They describe how clinical characteristics and diagnostic tests may be used to distinguish between these, and how steroidogenic biochemistry is evolving in this field. Therapeutic approaches are also included.

Li et al. from China present a systematic review and meta-analysis of the screening results for congenital adrenal hyperplasia involving 7.85 million newborns. The incidence of 1/23,024 was higher in males than females, possibly as a result of the gender imbalance in China, the greater attention paid to male infants resulting in a higher recall rate, or the diagnosis of ambiguous genitalia before or after birth in females, making screening unnecessary in girls.

With advancement in the genetic diagnosis of disorders of sex development, Bertelloni et al. outline a different approach to the investigation of 46XY DSD to the extensive, often repeated and invasive laboratory testing, by using advanced genetic technologies (next generation sequencing, whole exome sequencing, targeted CGH array) as the first line test after karyotyping and salt-loss exclusion which may result in a molecular diagnosis and guide more targeted biochemical investigations. A causative genetic diagnosis allows for accurate prognosis and recurrence risk.

Lucaccioni et al. review the current understanding of minipuberty and using this window as an opportunity for the diagnosis, and potentially treatment, of babies with DSD which could alter the natural history. They highlight the hormonal changes that occur and how minipuberty modulates neurobehavioral development.

Edwards and Harding review clinical aspects of transient neonatal hypoglycemia. They discuss pathophysiology, controversy over definitions of hypoglycemia, when and how to make blood glucose measurements, and use of glucose gel to prevent hypoglycemia. They highlight the need for evidence as to whether transient asymptomatic hypoglycemia is associated with brain injury, and if so, at what level or duration.

Chandran et al. report a family with a novel HNF4A mutation presenting with differing phenotypic presentations of glucose dysregulation. They describe an infant with diazoxide responsive hyperinsulinaemic hypoglycemia, who shares a novel HNF4A mutation with a sister who had transient neonatal hypoglycemia, and his father who developed diabetes at the age of 15. Implications of the genetic diagnosis on treatment and prognosis are discussed.

Beardsall reviews hyperglycemia with a focus on the preterm infant, and discusses pathogenesis, glucose insensitivity and insulin resistance, relative insulin deficiency, the clinical consequences of hyperglycemia, and its clinical management. The review provides a wealth of information on underlying mechanisms, which may be of particular value for neonatologists.

Beltrand et al. describe how the recent advances in neonatal diabetes mellitus can guide management and how the known genetic mutations define the pathophysiology by causing either abnormal β-cell function or abnormal pancreatic morphology. The authors provide a detailed clinical description of both the permanent and transient forms. The challenge of insulin therapy in maintaining normoglycaemia is discussed, as is the management of those with mutations in the K_{ATP} channel with suphonylureas to which the channel remain sensitive in 90% of cases. A helpful appendix provides practical advice on switching from insulin to glibenclamide, the oral suspension of which is licensed for children in the European Union.

In the review of current insights into disorders of calcium and phosphate in the newborn, Taylor-Miller and Allgrove examine the current understanding of fetal-to-neonatal mineral homeostasis mechanisms (calcium, phosphorus, magnesium) as well as vitamin D. Recent genetic discoveries have shed light on the pathophysiology of some causes of neonatal hypo and hypercalcemia. The presentation and management of bone fragility is discussed as well as the investigation and management of disorders of calcium and phosphorus homeostasis.

Saraff et al. describe an approach to neonatal bone disorders for clinicians, highlighting that early and accurate diagnosis in these rare disorders can be important for potentially life-saving treatment. The review includes structural bone defects, and bone mineralization defects, and an approach to diagnosis and management. This practical approach could be extremely useful for neonatal clinicians.

This series of articles was conceived with Dr. Paolo Ghirri, Associate Professor of Pediatrics at the University of Pisa, where he was the director of Neonatology at the Santa Chiara. Following his sudden and untimely death during the planning stage, we decided to dedicate the series to his life and work and made our contributors aware of this at the time of invitation.

AUTHOR CONTRIBUTIONS

Both authors contributed equally to this editorial and approved it for publication.

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This collection was conceived with Dr. Paolo Ghirri, and is dedicated to him. We are extremely grateful to our

contributors for providing such excellent manuscripts to produce the collection.

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 .

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CASE REPORT
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A Novel *HNF4A* Mutation Causing Three Phenotypic Forms of Glucose Dysregulation in a Family

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Maturity-onset diabetes of the young (MODY) classically describes dominantly inherited forms of monogenic diabetes diagnosed before 25 years of age due to pancreatic β-cell dysfunction. In contrast, mutations in certain MODY genes can also present with transient or persistent hyperinsulinemic hypoglycemia in newborn infants, reflecting instead β-cell dysregulation. Of the MODY genes described to date, only hepatocyte nuclear factor-4-alpha (*HNF4A*; MODY1) and hepatocyte nuclear factor-1-alpha *(HNF1A*; MODY3) mutations may result in a biphasic phenotype of hypoglycemia in early life and hyperglycemia in later life. We report a family with a novel *HNF4A* mutation with diverse phenotypic presentations of glucose dysregulation. The proband was a term, appropriate-for-gestational age male infant with symptomatic hypoglycemia on day 3 of life needing high glucose infusion rate to maintain normoglycemia. He was born to a non-obese and non-diabetic mother. Glucose regulation was optimized using diazoxide upon confirmation of hyperinsulinism. Cascade genetic screening identified the same mutation in his father and elder sister, but mother was negative. Father was diagnosed with Type 1 diabetes at 15 years of age that required insulin therapy. Proband's elder sister, born at term appropriate for gestational age, presented with transient neonatal hypoglycemia needing parenteral glucose infusion for a week followed by spontaneous resolution. The paternal grandparents were negative for this mutation, confirming a paternal *de novo* mutation and autosomal dominant inheritance in this family. This pedigree suggests that the presence of early-onset paternal diabetes should prompt molecular testing in infants presenting in the newborn period with diazoxide-responsive hyperinsulinemic hypoglycemia, even in the absence of maternal diabetes and macrosomia.

Keywords: maturity-onset diabetes mellitus, hepatocyte nuclear factor 4-alpha, hepatocyte nuclear factor−1- alpha, hyperinsulinemic hypoglycemia of infancy, congenital hyperinsulinism, diazoxide

INTRODUCTION

Maturity-onset diabetes of the young (MODY) is an acronym used to describe dominantly inherited forms of monogenic diabetes diagnosed before 25 years of age (1). MODY gene mutations have been described with clinically heterogeneous phenotypes (2). Among these, only *HNF4A* (MODY1) and *HNF1A* (MODY3) mutations on chromosomes 12 and 20 respectively, may result in a biphasic phenotype (3). *HNF4A* is an orphan receptor protein expressed in the liver, kidney, gut, and pancreatic β-cells (4). Mutations in *HNF4A* may lead to biphasic presentations, characterized by transient or persistent HH in infants, and diabetes in young adults. *HNF4A* mutations are a less common cause of MODY (10%) than glucokinase *(GCK)* (30–50%) and *HNF1A* (30–50%). Among infants with *HNF4A* mutations, 56% are macrosomic and 15% encounter neonatal hypoglycemia (5). The prevalence of neonatal hypoglycemia is similar regardless of parental *HNF4A* inheritance and persistent hypoglycemia is independent of gestational glucose control (6). Hyperinsulinemic hypoglycemia (HH) is characterized by inappropriate insulin secretion while hypoglycemic and the need for high glucose infusion rate (GIR) requirements (>8 mg/kg/min) to maintain normoglycemia (3.5–5.9 mmol/L) in newborn infants beyond 48 h of life. HH infants have inappropriate insulin and/or C-peptide levels despite the presence of hypoglycemia, hypoketonemia and hypofattyacidemia. HH is linked to mutations in at least eight genes (*ABCC8, KCNJ11, GLUD1, GCK, HADH, SLC16A1, HNF4A, HNF1A*) that alter β-cell function (7). Unlike the majority of mutations involving the K_{ATP} channel, *HNF4A* mutations causing HH respond well to diazoxide (8). We present a family with a novel *HNF4A* mutation identified from a proband presenting with symptomatic hypoglycemia. The family members were found to have contrasting phenotypic presentations of glucose dysregulation in spite of having the same mutation. We present this pedigree to demonstrate that a history of paternal diabetes is as important as a history of maternal diabetes, and relevant in pediatric history-taking when managing infants at-risk of hypoglycemia.

CASE PRESENTATION

Pedigree Report

A healthy male infant weighing 3,592 gm was born to non-consanguineous parents at 37+5 weeks gestation and discharged uneventfully on day 2 of life. Both parents are of Malay ethnicity. Maternal health during pregnancy and oral glucose tolerance test results were normal. There was no maternal family history of diabetes. On day 3 of life, this infant was admitted for treatment of neonatal jaundice. While on phototherapy he was noted to be apneic and cyanosed with low plasma glucose (1.8 mmol/L). Resuscitation involved mini bolus intravenous 10% dextrose and continuous glucose infusion. As the response was inadequate, GIR was graded up to 16 mg/kg/min before glucose levels normalized.

Physical examination of the infant was unremarkable. Upon reduction of GIR in a controlled setting, critical blood tests were obtained that showed detectable C- peptide [1.7mcg/L] and insulin [10.4 mU/L] when plasma glucose was 1.6 mmol/L. Along with suppressed blood ketones of 0.2 mmol/L, these indicate inappropriate insulin production during hypoglycaemia, which fulfilled the diagnostic criteria for HH. Serum cortisol was 494 nmol/L and GH level was 13.4 ug/L, showing adequate pituitary response. Septic and inborn error of metabolism screens were negative. On day 7 of life, he was started on diazoxide, 5 mg/kg/day in divided doses, with normoglycemia achieved after titrating diazoxide up to 10 mg/kg/day on day 19 of life. Hydrochlorothiazide was added to counteract the salt and water retaining side effects of diazoxide. Thereafter, his GIR was weaned over 5 days and he remained normoglycemic on full oral feeds. Before discharge while on diazoxide, he passed a 6-h safety fast study, to reassure of his ability to maintain glucose levels during inadvertent fasting periods at home. Glucose monitoring continued at home. The absolute diazoxide dose was maintained, allowing weight-based reduction of the dose toward 24 months of age. At 36 months, diazoxide was stopped and he underwent and passed a resolution fasting study. Growth and neurocognitive development are currently appropriate for age.

Genetic testing for hyperinsulinism was performed at Exeter, UK. Analysis of coding and flanking intronic regions of the *KCNJ11*, *ABCC8,* and *HNF4A* genes by Sanger sequencing was done. Both *KCNJ11* and *ABCC8* genes were normal. A novel heterozygous *HNF4A* missense mutation, p.Asp345Tyr (c.1033G>T) was identified. Cascade family screening identified the same *HNF4A* mutation in his father and elder sister; whereas his mother and paternal grandparents were negative (**Figure 1**). This confirmed that the proband's father has a *de novo* mutation, consistent with MODY rather than type 1 diabetes.

The proband's father was diagnosed with diabetes at age 15 years and treated with insulin. He was obese from early childhood, even though there was no history of diabetes in his parents or siblings. Details of initial management prior to his transfer to tertiary diabetes care are unavailable. At age 33 years, his glycated hemoglobin (HbA1c) was 12%, fasting glucose 18.3 mmol/L, C-peptide 270 pmol/L (364-1655), glutamic acid decarboxylase (GAD) autoantibody and islet-cell autoantibody tested negative. Diabetes control was suboptimal due to poor adherence. On Metformin 850 mg twice daiy (BD) and basal-bolus insulin therapy, HbA1c ranged from 6.9 to 10% over the next 5 years. At age 38 years, he was reassessed following his son's genetic diagnosis. While on Metformin 850 mg BD, subcutaneous (SC) Glargine 16u BD and SC Glulisine 10–14u thrice daily (TDS), HbA1c was 9%, fasting glucose 16.5 mmol/L and C-peptide 481 pmol/L. He measured 1.54 m, weighed 86 kg, giving a BMI of 36.3 kg/m^2. Given his reasonable insulin reserve, sulphonylurea therapy was initiated to determine if insulin doses could be reduced without compromising glucose control. Ambulatory glucose profiles were conducted over a 2-week period—in the first week, he was on his usual treatment regimen while in second week, sulphonylurea was added (**Figure 2**). He responded to up-titrated doses of Glibenclamide with reduction of basal insulin from 32 to 8 units daily, while maintaining similar glucose profiles. Over these 2 weeks, the composite ambulatory glucose profile showed average glucose of 9.6 mmol/L, giving

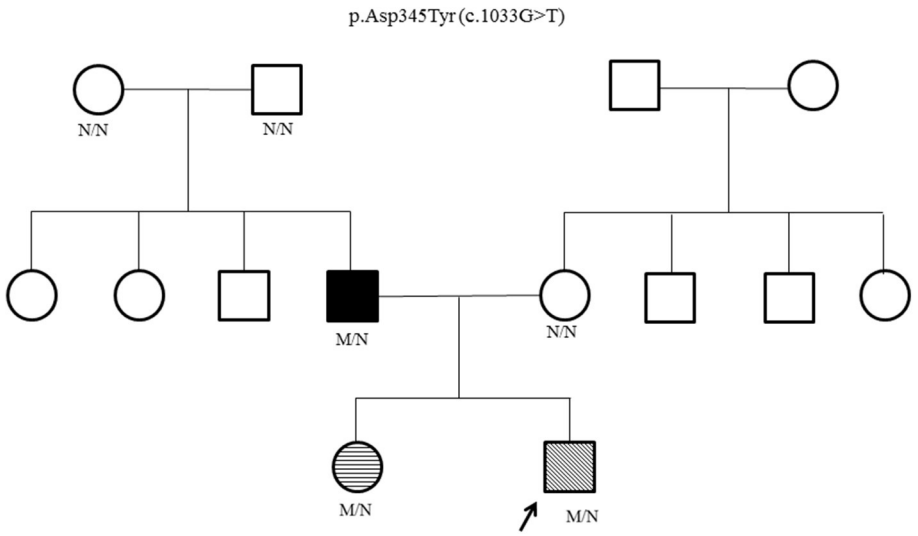

FIGURE 1 | *HNF4A* family pedigree. Circles (females) and squares (males). Arrow marks the Proband. Filled symbol indicates MODY (father); unfilled symbol indicates no history of glucose dysregulation. Diagonal hatching denotes persistent HH (proband). Transverse hatching indicates transient hypoglycemia (sister). M/N = heterozygous *HNF4A* genotype; N/N = normal genotype.

an estimated HbA1c of 7.7%. His current medication doses are Glibenclamide 7.5 mg BD, Metformin 850 mg BD, SC Glargine 8 units every night (ON) and SC Glulisine 10u BD. After glibenclamide was added and the basal insulin dose was reduced, there was improvement in his fasting glucose levels from 11-13 mmol/L to 5-6.4 mmol/L. Despite subsequent increments in sulphonylureas, he required prandial (albeit decreased) doses of glulisine for persistent postprandial hyperglycemia. His total daily insulin requirements decreased from 1 to 0.5 u/kg/d. The incomplete response to sulphonylureas was likely due to progressive defect in beta cell β-cell dysfunction after having had diabetes for 23 years. His most recent BMI was 36.4 kg/m^2.

The proband's 8 year old sister who was born term, appropriate for gestational age (birth weight, 2835 g) had transient hypoglycemia during neonatal period. Work up for sepsis and inborn error of metabolism screen were negative. However, her phenotype was mild and she required intravenous dextrose (highest GIR 7.6 mg/kg/min on day 4 of life), before gradual increase in feeds normalized her blood glucose levels by day 7 of life. She has appropriate growth and remains in a mainstream school (**Figure 3**).

Family's Perspective

Upon receiving news of the genetic diagnosis, the proband's father was hopeful for better control with reduced insulin. Although his endocrinologist was able to use this information to adjust his treatment regimen, he experienced difficulty making lifestyle changes which limited his glucose control and prevented further reduction of his medications. The proband's mother was more concerned about the implications of the diagnosis on her children. She was particularly happy that the proband did not require long-term Diazoxide therapy, although she was concerned of the risk of diabetes in both her children. The proband's paternal grandparents expressed that the exact diagnosis makes them aware of the risk of childhood-onset diabetes. They clearly indicated their hope for their grandchildren to remain healthy and not to develop obesity and childhood-onset diabetes like their son (the proband's father) did.

DISCUSSION

We describe a pedigree where a heterozygous novel *HNF4A* mutation was identified in 3 individuals of a 2 generation family. Each of these individuals were phenotypically distinct—the proband had persistent HH, his sister had transient hypoglycemia, while his father had juvenile-onset MODY. The paternal grandparents tested negative, confirming a spontaneous *de novo* mutation, followed by dominant inheritance. Identification of the underlying genetic etiology allowed for a molecular diagnosis of the proband, clarified the paternal phenotype as MODY instead of type 1 diabetes and facilitated his improved diabetes management.

Up to 80% cases of diabetes due to MODY gene mutations are misclassified as type 1 or type 2 diabetes, leading to inappropriate medical therapy (9). The diagnosis of MODY requires molecular confirmation (10). In retrospect, precise molecular diagnosis of the diabetes in the proband's father would have permitted close fetal monitoring for macrosomia and appropriate postnatal glucose surveillance. Mutations in *HNF4A* are reported to be highly penetrant with 50% of carriers developing diabetes by age 30 years, whereas 60% with *HNF1A* mutations present by 25 years (11). The father of the proband was diagnosed with diabetes at age 15 years, expressing the highly penetrant nature of this novel *HNF4A* mutation. Infants who inherit *HNF4A* have significantly increased birth weight, with more than half having

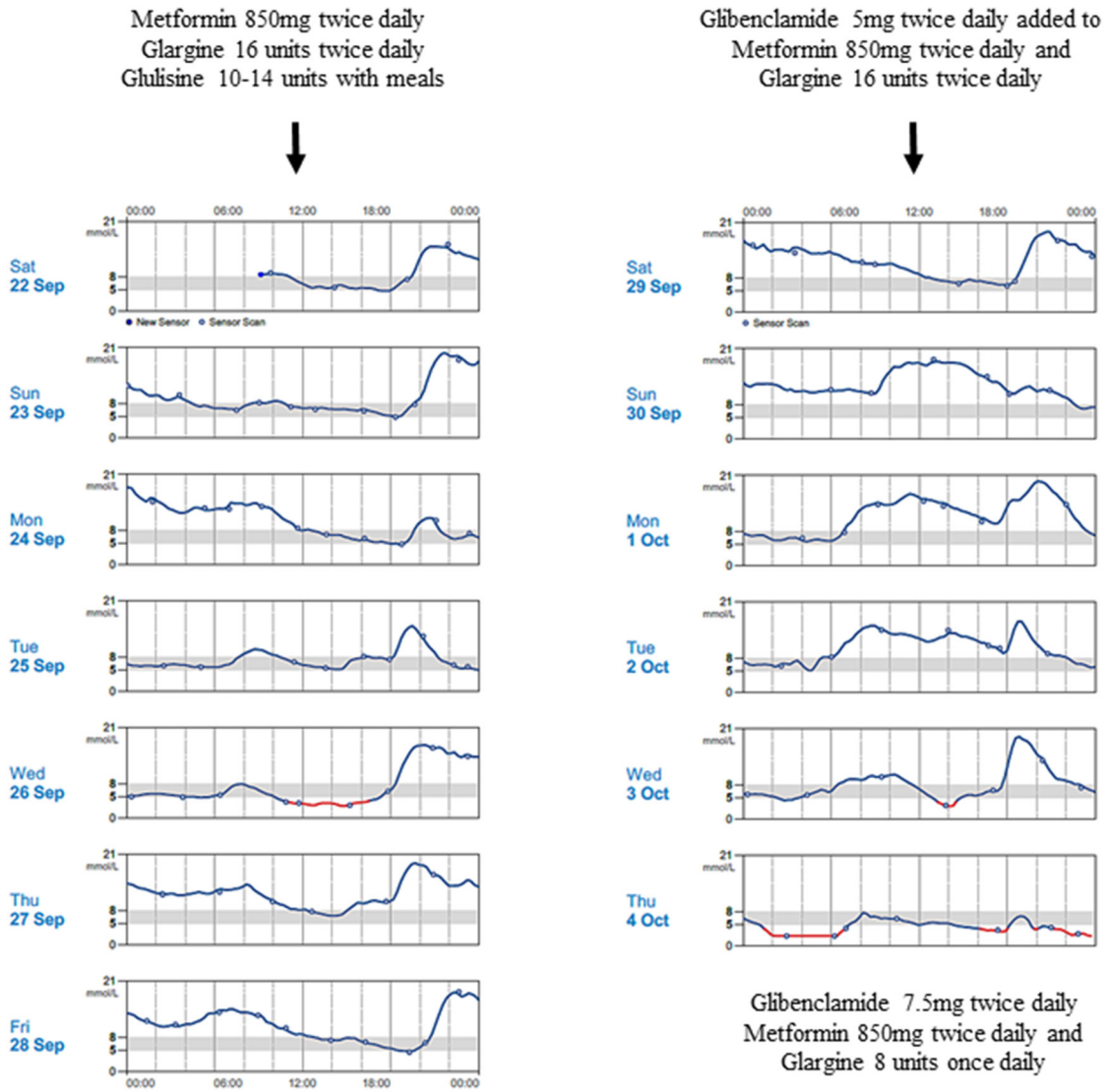

FIGURE 2 | Continuous blood glucose patterns of the proband's father, capturing his daily glucose variability over 2 weeks, while he was on Metformin 850 mg twice daily, Glargine 16 units twice daily and Glulisine 10–14 units thrice daily (first week); and demonstrating improved fasting glucose levels despite progressive reduction in Glargine doses after Glibenclamide was progressively introduced (second week).

macrosomia. The risk of macrosomia is higher in maternally-inherited mutations (64%) compared to paternal inheritance (46%), due to the additional effect of hyperglycemic intrauterine milieu (6). The paternal inheritance pattern in this pedigree may explain the absence of macrosomia in both his offsprings.

HNF4A mutations are the third most common genetic cause of diazoxide-responsive HH, (8) however the mechanism by which these mutations cause insulin excess in fetal and neonatal life and insulin deficiency later in life is unclear. Heterozygous loss-of-function mutations in *HNF4A* may cause either transient or persistent HH (6, 12). Current evidence supports a reduction in expression of inward rectifying potassium channel subunit (Kir 6.2) and/or reduction in the levels of peroxisome proliferator-activated receptor alpha (PPARα), causing inappropriate insulin secretion and resulting in HH in newborn period (13, 14). Low levels of PPARα have been reported in *HNF4A* deficient β-cells, resulting in the accumulation of lipids and thereby increasing cytosolic long-chain acyl-CoA levels, signaling insulin release. Long-term exposure of β-cells to elevated concentrations of fatty acids causes β-cell dysfunction leading to diabetes (15). Other suggested theories for the dual phenotype in *HNF4A* mutations include variance in *HNF4A* dependent temporal gene expression, β-cell exhaustion from hypersecretion in fetal life and infancy and malfunction of transcription factors that sustain β-cell function in pancreatic islets (7, 16, 17). MODY responds well to low-dose sulfonylurea, maintaining the glucose profile even after 3 decades (16, 18). In individuals with a *HNF4A*

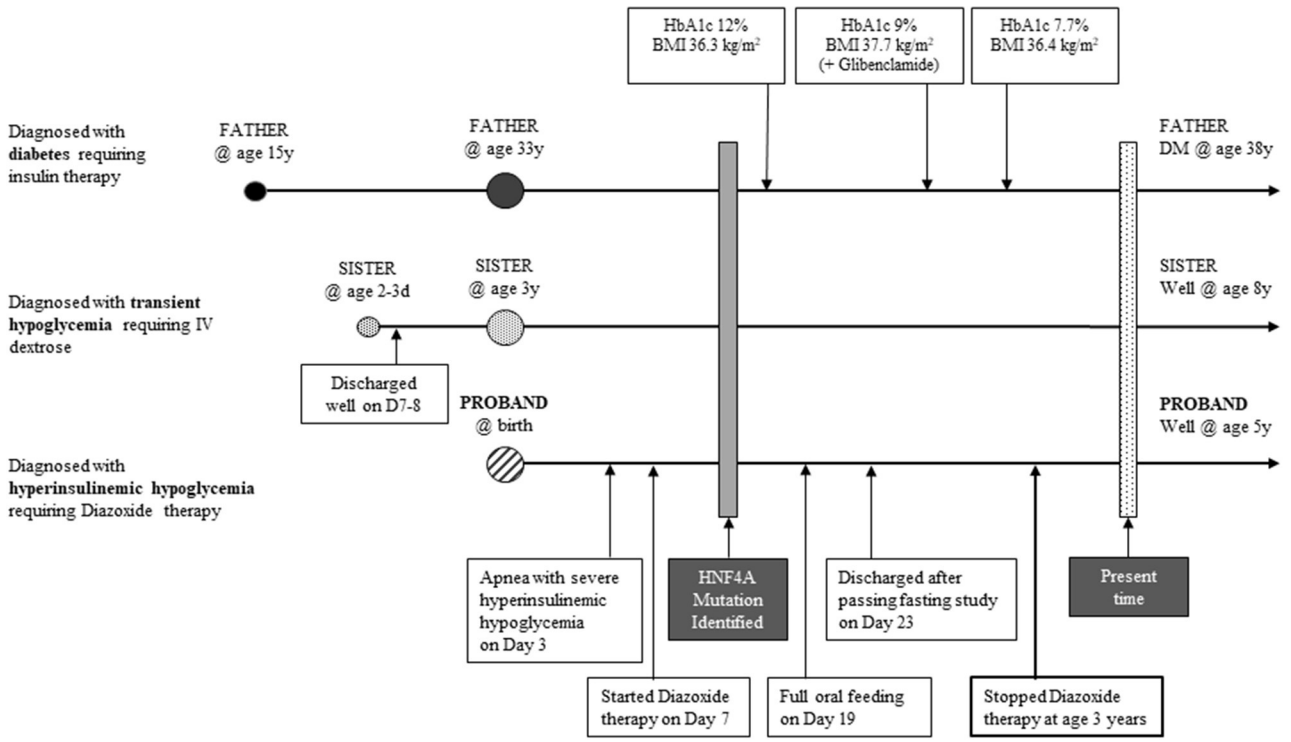

FIGURE 3 | Timeline depicting the three phenotypes of this pedigree.

mutation, as the β-cell dysfunction is progressive with age, insulin treatment may eventually be required. If the proband's father had received an early genetic diagnosis, he may have benefitted from oral sulfonylurea instead of insulin. Secondary sulphonylurea failure has been described to occur in 3 to 25 years following diagnosis/treatment in transcription factor linked-MODY patients (19). Unlike *GCK* mutations, patients with diabetes due to *HNF4A* and *HNF1A* mutations are at increased risk of micro and macrovascular complications, (6, 9, 18, 20) and therefore require early and sustained glucose control.

Diazoxide remains the first line of medical treatment for HH. Diazoxide response in HH due to a *HNF4A* mutation is adequate but the treatment period may vary from months to years (9). In this proband with a novel *HNF4A* mutation, glucose levels were controlled with moderate doses of diazoxide weaned over 3 years. Following cessation of therapy, resolution of HH was confirmed with a fasting study. As there is potential of developing MODY, glucose tolerance testing is planned for the proband and his sister.

The strength of this case study lies in the full phenotypic characterization of the proband and cascade genetic testing in his family. However, type 1 diabetes management details of the father are unavailable prior to the proband's diagnosis. The proband's sister was not evaluated for hyperinsulinism as she did not meet the criteria for HH.

In conclusion, we present a family with a novel *HNF4A* mutation having 3 phenotypic presentations across 2 generations. This case pedigree supports *HNF4A* gene sequencing for infants presenting in the newborn period with diazoxide-responsive HH and paternal diabetes, even in the absence of maternal pre or gestational diabetes and fetal macrosomia. Those with prior molecular diagnosis of *HNF4A* mutation should be monitored for early diagnosis of sulfonylurea-sensitive diabetes, to allow earlier intervention and treatment. Overall, an early molecular diagnosis of *HNF4A* MODY can guide a change in therapy from insulin to sulfonylurea, improving the lifestyle and quality of life for both patients and their families.

DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in the HNF4A mutation details have been deposited in the Decipher database (https://decipher.sanger.ac.uk/). The raw dataset can however be used to identify individuals and so cannot be made openly available. Access to data is open through collaboration. Requests for collaboration will be considered following an application to the Genetic Beta Cell Research Bank (https://imsva91-ctp.trendmicro.com:443/wis/clicktime/v1/query?url=https%3a%2f%2fwww.diabetesgenes.org%2fcurrentresearch%2fgenetic%2dbeta%2dcell%2dresearch%2dbank%2f&umid=1C0AE20F-9E54-0305-9291-12237D74D356&auth=6e3fe59570831a389716849e93b5d483c90c3fe4-5a72b0906f4668ba59b7efa43cf143736ad6be6c). Contact by email should be directed to the lead nurse, Dr. Bridget Knight (b.a.knight@exeter.ac.uk).

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

SC and FY treated the infant, wrote the manuscript, and performed the final edits. WH treated the father of the index case and wrote the paternal case section. VR reviewed and revised the manuscript critically for important intellectual content. SF and KH performed the genetic testing for the pedigree, contributed to the genetic section of the manuscript and reviewed the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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A Detailed Analysis of the Factors Influencing Neonatal TSH: Results From a 6-Year Congenital Hypothyroidism Screening Program

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Background: Neonatal thyrotropin (TSH) on dried blood spot (DBS), the most common screening strategy for primary congenital hypothyroidism (CH), is influenced by numerous factors that may hinder a true CH diagnosis. A second test can thus be performed to clarify the initial findings, although its application varies among screening programs.

Objectives: The aim of this study was to evaluate the effect of maternal and neonatal factors on neonatal TSH levels and offer practical screening recommendations.

Methods: We retrospectively analyzed screening data of 62,132 neonates born in Abruzzo, an Italian region considered mildly iodine deficient, between 2011 and 2016. We then performed a multiple linear regression to model the relationship between TSH (the dependent variable) and 13 independent variables extracted from blood collection cards.

Results: Most neonates (53,551 of 62,132, 86%) had normal TSH and no clinical indications for a second screening. A minority (1,423, 2.3%) had elevated TSH in the initial DBS, which was confirmed in 97 cases (7%) on a second screen. The remaining neonates (6,594, 10.6%) had a normal initial TSH but underwent a second test in accordance with screening protocols, and were found to have delayed TSH elevation in 23 cases (0.4%). Those 120 newborns (97 + 23), considered highly suspicious for primary CH, were referred to a pediatrician for confirmatory testing and excluded from subsequent analysis of factors influencing TSH levels. Sex (β regression coefficient, β = 1.11 female to male, 95% CI 1.09, 1.12) and age at collection (β = 0.78 day 5 to days 2–3, 95% CI 0.74, 0.83) affected neonatal TSH, suggesting the utility of specific nomograms. In addition, prematurity (β = 0.85 term to preterm, 95% CI 0.80, 0.91), dopamine use (β = 0.71, 95% CI 0.62, 0.81), and birth weight (β = 1.40 normal vs. very low, 95% CI 1.05, 1.89) strongly influenced neonatal TSH.

Conclusions: Neonatal TSH is influenced by several factors supporting the delineation of local sex- and age-adjusted TSH cutoffs, and the universal adoption of a second TSH test in neonates at risk of missed primary CH diagnosis.

Keywords: newborn screening, congenital hypothyroidism, thyroid stimulating hormone (TSH), thyroid diseases, preterm

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INTRODUCTION

Congenital hypothyroidism (CH) indicates the deficiency of thyroid hormones at birth, a deficiency that if unrecognized and untreated leads to severe intellectual disability and growth retardation. To recognize and promptly treat CH, a neonatal screening program was universally introduced, in Italy starting in 1977 and reaching full coverage in the early 1990s (1). The Italian CH screening is performed, as of December 31, 2016, by 26 regional and inter-regional clinical laboratories that screen newborns using either Dried Blood Spot (DBS) TSH (20 of 26, 77%), or both DBS TSH and total thyroxine (6 of 26, 23%) (2). Screening data are then reported to the Italian National Registry of Infants with Congenital Hypothyroidism, an office established by the Italian Ministry of Health in 1987.

The threshold value to determine whether TSH is elevated (hence the neonate at risk of primary CH or not) varies across Italian screening centers, with a range comprised between 6 and 12 mU/L (2). This threshold value has decreased over the years, resulting in a greater detection of primary CH cases, mostly mild (3).

Even when the initial TSH value is normal, recent guidelines (4) suggest collecting a second DBS specimen if the neonate belongs to categories known to delay the TSH elevation so as not to miss possible primary CH diagnoses. These categories include prematurity (i.e., gestational age < 37 weeks), low and very low birthweight, twin delivery, congenital malformations, and chromosomal abnormalities such as down syndrome, blood transfusions, dopamine, total parenteral nutrition, or other conditions requiring admission to the neonatal intensive care unit (4, 5). Despite the guidelines, however, a lack of uniformity in screening programs for preterm, low, and very low birthweight neonates does occur, as recently reviewed in (6). Most authors advocate a second screening strategy, while others suggest either lowering the screening cutoff, using gestational age-adjusted cutoff, or testing for both TSH and T4 (6). It also remains unclear whether other factors such as sex, season of birth, and maternal history of autoimmune thyroid diseases should be considered in screening protocols.

The aim of this study was to evaluate the effect of maternal and neonatal factors on neonatal TSH levels and offer practical recommendations to improve current screening algorithms for primary CH.

MATERIALS AND METHODS

Study Population and Screening Protocol

We retrospectively analyzed data of all babies born in Abruzzo between January 1, 2011 and December 31, 2016 and screened for primary CH by DBS TSH. The Abruzzo CH screening program, which began in 1994, is housed in Chieti at the Center of Sciences on Aging and Translational Medicine (CeSI-MeT). In most cases, blood was collected by heel prick between 48 and 120 h of life, spotted onto collection cards known as Guthrie cards (Whatman 903, Expertmed SRL, Verona, Italy), and then mailed to the CeSI-MeT laboratory. When newborns were transferred to another hospital, however, blood samples could also be collected before 48 h. Cards of poor quality and/or containing insufficient blood were considered "*inadequate*" for the assay and prompted the request of a new sample.

Cards originated from 12 hospitals (Atri, Avezzano, Chieti, L'Aquila, Lanciano, Ortona, Penne, Pescara, Sant'Omero, Sulmona, Teramo, and Vasto) located in the four provinces (Chieti, Pescara, Teramo, L'Aquila) of Abruzzo, a region that is still considered mildly iodine deficient (7). The following information was extracted from the collection cards: sex, date of birth, province of birth, age at blood collection, prematurity (reported as gestational age < 37 weeks or not), birthweight, dopamine, total parenteral nutrition, blood transfusions, malformations, twin delivery, pre-gestational history of thyroid disease, and/or use of anti-thyroid drugs during pregnancy. Unfortunately, gestational age on collection cards was reported as a categoric variable (i.e., gestational age <37 or not), limiting the possibility to define the newborn as small or appropriate for gestational age. Furthermore, cards did not include information about mode of delivery, APGAR score, hematocrit, administration of glucocorticoids, and maternal iodine status.

TSH was measured using an automated time-resolved Fluoro-Immuno-Assay that uses a monoclonal antibody directed against the β subunit of human TSH (AutoDELFIA hTSH, Perkin Elmer, Waltham, MA). According to the manufacturer, the analytical sensitivity is 2 mU/L, although values comprised between 0.5 and 2 mU/L were reported by the analyzer. The screening program used a cutoff value of 7 mU/L to distinguish normal from elevated TSH, a value approximating the 98th percentile of the TSH distribution observed in term and normal weight neonates who do not have congenital hypothyroidism.

Neonates with DBS TSH values <7 mU/L were considered negative for primary CH and underwent no further testing. Those with TSH ≥ 7 mU/L were recalled for a second DBS collection; if TSH elevation was confirmed, they were considered highly suspicious for primary CH and referred to pediatrician for confirmatory testing. Neonates with TSH <7 mU/L in the initial sample but belonged to "at risk" categories (preterm, low birthweight, twins, malformations, recipients of blood transfusions, dopamine, total parenteral nutrition, and/or born to mothers with a history of autoimmune thyroid disease) underwent a routine second TSH screening after 15 days of age.

Study Outcomes and Statistical Analysis

DBS TSH was the main outcome variable of the study and was related to the following 13 covariates: sex (male or female), calendar year of birth (2011, 2012, 2013, 2014, 2015, or 2016), season of birth (winter, spring, summer, or fall), province of birth (Chieti, Pescara, Teramo, L'Aquila), age at blood drawing (in days), use of dopamine (yes or no), total parenteral nutrition (yes or no), blood transfusions (yes or no), malformations (yes or no), maternal history of autoimmune thyroid disease (yes or no), twin delivery (yes or no), prematurity (yes or no), and birthweight (normal birthweight, NBW, 2,500–4,500 g, or abnormal birthweights). Prematurity was defined as pregnancy duration <37 weeks.

Abnormal birthweights were classified according to the four World Health Organization categories (8): high birth weight, (HBW, >4,500 g), low birth weight (LBW, 2,500–1,500 g), very low birth weight (VLBW, 1,000–1,499 g), and extremely low birth weight (ELBW, <1,000 g).

De-identified data were entered into a FileMaker database (FileMaker Pro Advanced 14.0.1, Inc., Santa Clara, CA, USA) and then analyzed using the statistical software Stata (Stata 15.1, College Station, TX, USA). We excluded from the analysis of factors influencing TSH levels, blood samples that were inadequate for the TSH assay, samples not accompanied by information about age at collection and/or birthweight, samples coming from babies born outside of the Abruzzo region, samples collected beyond 15 days of age, and samples that were classified as "highly suspicious for primary CH."

Data were described using mean and standard deviation for normally distributed quantitative variables, median, and interquartile range for non-normally distributed quantitative variables, and frequencies and percentages for qualitative variables. Monthly birth rate was calculated by dividing the number of births in each month by the total Abruzzo population in the corresponding year, this latter information obtained from the Italian National Institute of Statistics.

The neonatal TSH percentile charts were created by calculating in healthy neonates the 10, 25, 50, 75, 90, 95, 97.5, and 99th percentiles of TSH according to sex and neonatal age. Arithmetic TSH values were transformed to a natural logarithm scale to approximate the normal distribution.

We initially performed a series of simple linear regressions where the log-transformed TSH was related individually to the 13 covariates. We then used multiple linear regression to model the log-transformed TSH based on the combination of all covariates.

In this model, prematurity, total parenteral nutrition, dopamine administration, and blood transfusion were combined into one regressor called infant factors; whereas history of maternal autoimmune thyroid disease and twin-delivery into a regressor called maternal factors. The final model, therefore, included eight covariates: sex, calendar year of birth, season of birth, province of birth, age at blood drawing, birthweight, infant factors, and maternal factors. A ninth covariate was created by multiplying prematurity by birthweight to assess their interaction.

Normal plot of residuals was used to check the normality of the residual distribution. Linearity and equal variance (homoscedasticity) of residuals were checked by examining the plot of standardized residuals. Co-linearity between predictors was tested using the test of variance inflation factor.

RESULTS

Outcomes of the CH Screening

Between January 1, 2011 and December 31, 2016, a total of 71,743 collection cards were received at the Abruzzo regional screening center for primary CH, corresponding to 62,132 newborns. Of them, 53,551 newborns (86.2%) had a TSH value <7 mU/L and were therefore considered negative for primary CH (**Figure 1**).

In 1,423 newborns TSH was ≥7 mU/L in the initial screening, prompting the request of a second DBS sample, thus, yielding a recall rate of 2.3%. The second TSH measurement was abnormally elevated in 97 of 1,423 babies (7%, **Figure 1**) who were therefore referred to a pediatric endocrinologist for confirmatory testing ("highly suspicious for primary CH").

In 6,594 newborns (10.6%) the TSH, although normal in the initial screening, underwent a second test because of clinical features that made them at higher risk of having a missed diagnosis of primary CH (*negative* "*at risk*," **Figure 1**). Of them, 23 (0.4%) have elevated TSH on second screen and were referred to a pediatric endocrinologist for further investigation.

Of the referred 120 (97 + 23) newborns, 109 (89 + 20) had abnormal serum T4 and TSH values on confirmatory laboratory tests and initiated levothyroxine (L-T4) replacement therapy, whereas 11 (8 + 3) had normal serum T4 and normal or slightly elevated TSH, thus were classified as having "transient TSH elevation" and underwent periodic follow-up (**Figure 1**). The first group of newborns could not be distinguished from the latter group based on the 13 covariates analyzed in the study.

A few newborns (564 of 62,132, 0.9%) had cards of poor quality and/or containing insufficient blood ("*inadequate*"), which prompted the drawing of a second blood sample. At the second testing, the majority of them (98.9%) had normal TSH values and were considered negative for primary CH.

Overall, the period prevalence of primary CH in this 6-year interval was 0.17% (109 of 62,132, **Figure 1**). This value corresponded to an incidence of primary CH of 1 case every 570 births. The second screening performed in the "negative at risk" group identified three additional cases for 10,000 births. No clinical data were available to distinguish transient from permanent primary CH.

General Characteristics of the Study Population

Of the total 62,132 screened newborns, 1,315 (2.1%) were excluded from further analysis of factors influencing TSH levels because of a lack of information about sex (6, 0.01%) or birthweight (569, 0.9%), had an age > 15 days at the first blood drawing (35, 0.06%), were born outside Abruzzo (21, 0.03%), or lacked TSH value because the collection card was unsuitable for the assay (564, 0.9%). In addition, we also removed from the further analysis of factors influencing TSH levels the 120 babies that were confirmed to have elevated TSH, and thus classified as "highly suspicious for primary CH," to avoid the skewness that would derive from the elevated TSH values that are found in babies with thyroid dysgenesis or dyshormonogenesis (**Supplementary Figure 1**). The remaining study population included 60,817 newborns (**Supplementary Table 1**). Male newborns (31,753, 52.2%) were slightly more numerous than females (29,064, 47.8%; **Supplementary Table 1**). Summer months recorded the highest natality (**Supplementary Table 1**), with peaks in August and September (**Figure 2A**). Neonatal TSH was highest in winter and lowest in summer months during the

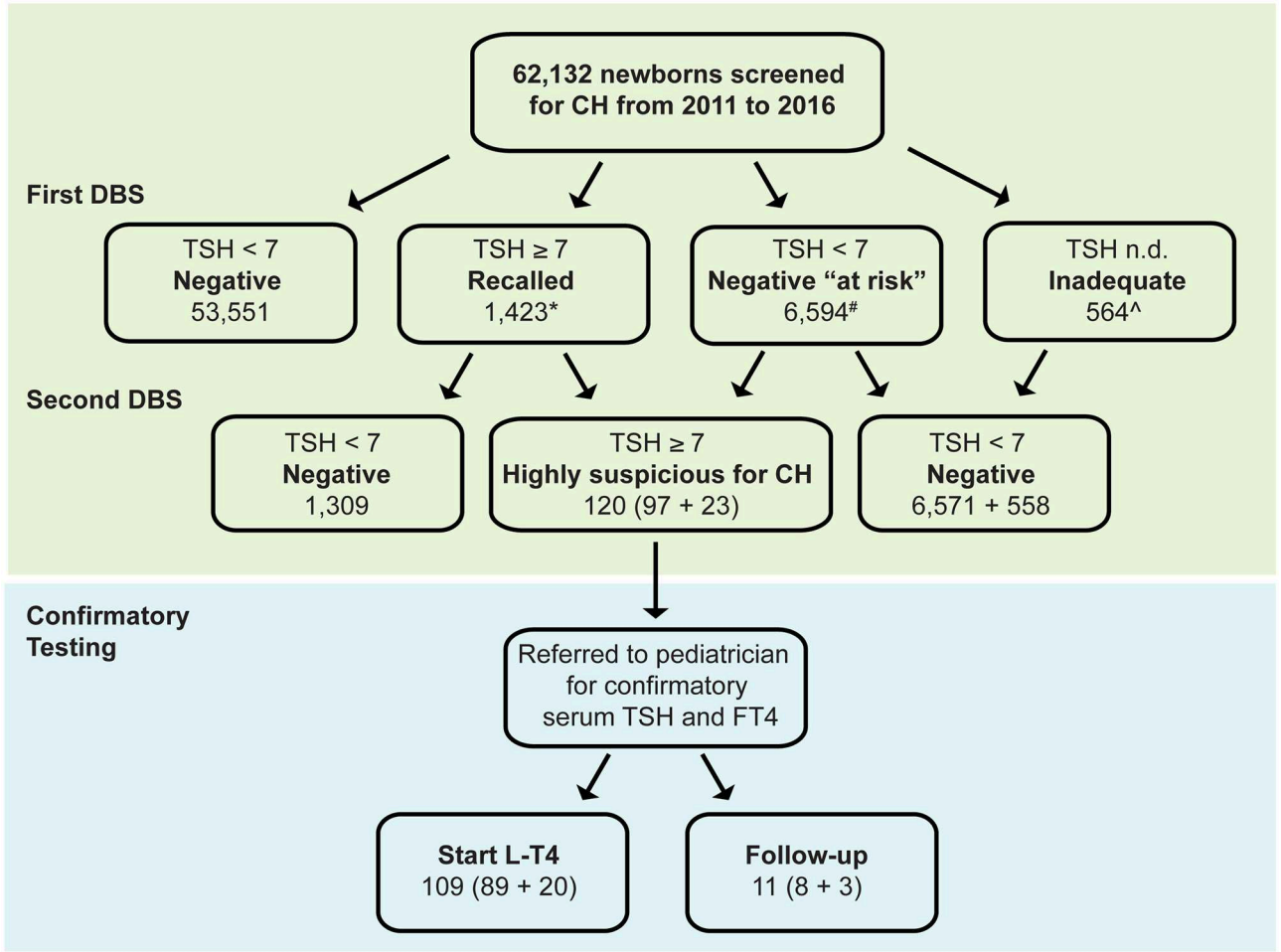

FIGURE 1 | Distribution of TSH results at the initial (first dried blood spot, DBS) and subsequent testing in 62,132 babies born in Abruzzo (Italy) between January 1, 2011 and December 31, 2016. DBS, dried blood spot, *17 lost to follow-up, ^6 lost to follow-up, #Neonates belonging to the following categories: preterm, low birthweight, twins, malformations, blood transfusions, dopamine, total parenteral nutrition, maternal thyroid disease CH Congenital hypothyroidism, L-T4 Levothyroxine.

6-year study period (**Figure 2B**). The province of Chieti had the greatest number of births (21,837 of 670,818, 36%), followed by Pescara (13,701, 23%), Teramo (11,344, 19%), and L'Aquila (13,935, 23%) (**Supplementary Table 1**).

The median age at blood collection was 3 days (IQR 1), with the vast majority (98%) of newborns tested between the 2nd and 4th day of age (**Figure 2C**). The median body weight at birth was 3,280 g (IQR 610, **Supplementary Table 1**).

About 4% of the newborns were preterm, 0.2% treated with dopamine, 0.3% received total parenteral nutrition, 0.4% received blood transfusions, 0.3% had malformations, 1.2% were born to mothers with autoimmune thyroid disease, and 3.2% were twins (**Supplementary Table 1**).

Individual Influence of the Risk Factors on Neonatal TSH Levels

When the 13 covariates were analyzed individually by simple linear regression for their influence on neonatal TSH (**Supplementary Table 2**, **Figure 2**, **Supplementary Figure 2**), the most notable effects were seen with prematurity, birthweight (**Figure 2**), sex, neonatal age at blood collection, blood transfusions, dopamine administration, and total parenteral nutrition (**Supplementary Figure 2**). Prematurity was associated with significantly lower TSH levels, with an average value of 1.1 mU/L as compared to 1.8 mU/L in term babies ($p < 0.0001$, **Figure 2D**). Birthweight strongly influenced the levels of TSH, which decreased in a dose-dependent fashion from high to extremely low birthweight (**Figure 2E**). The effect of birthweight on TSH, however, was modified by prematurity. In particular, term infants with VLBW had higher TSH values than those with NW, whereas preterm with LBW or VLBW had lower TSH values than preterm with a normal birthweight (**Figure 2F**). Sex and neonatal age at sample collection also markedly affected TSH levels, which declined from a median of 3.5 mU/L in males and 2 mU/L in females on day 0–1.3 mU/L in both genders on day 4, 5, and 6 (**Supplementary Figure 1A**).

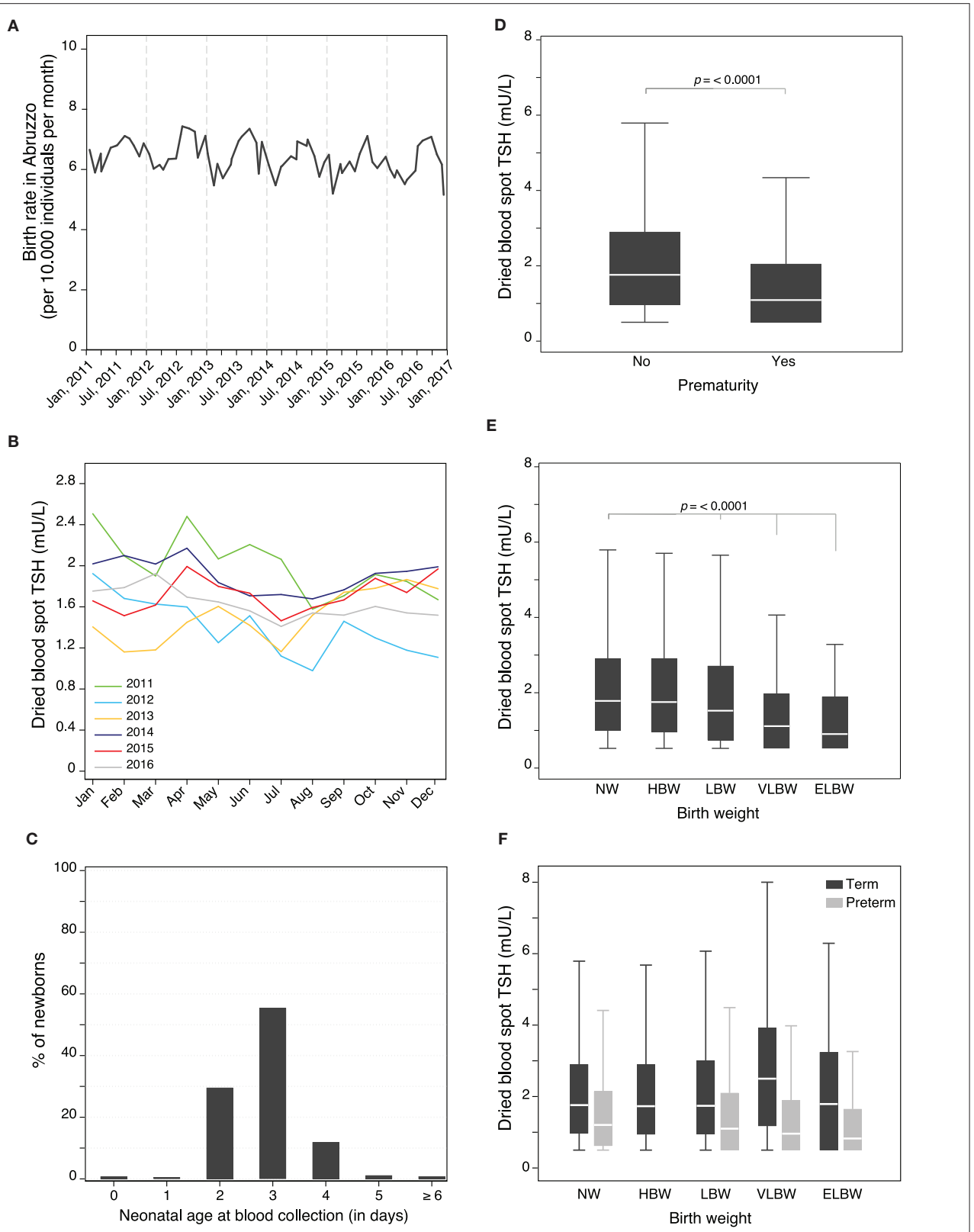

FIGURE 2 | (A) Monthly birth rate in Abruzzo (Italy) between January 1, 2011 and December 31, 2016. **(B)** Seasonal changes in neonatal TSH during the 6-year study period. **(C)** Distribution of newborns according to their neonatal age (in days) at blood collection. Individual influence of prematurity **(D)**, and birth weight **(E)** on neonatal TSH levels. Influence of birth weight on neonatal TSH levels adjusted for prematurity **(F)**.

TABLE 1 | Multiple linear regression analysis of the factors affecting neonatal TSH.

	ß	Standard error	(95% Confidence Interval)		*p*-value
$ß_0$	3.54	0.12	3.31	2.3.80	<0.0001
Sex, Male (Female ref)	1.11	0.01	1.09	1.12	<0.0001
Neonatal age at blood collection (Day 2–3 ref)					
Day < 2	1.49	0.04	1.42	1.57	<0.0001
Day 4	0.80	0.01	0.79	0.82	<0.0001
Day 5	0.78	0.02	0.74	0.83	<0.0001
Day ≥ 6	0.80	0.03	0.75	0.85	<0.0001
Season of birth (Winter ref)					
Spring	1.00	0.01	0.98	1.01	0.817
Summer	0.87	0.01	0.86	0.89	<0.0001
Fall	0.98	0.01	0.95	0.98	<0.0001
Infant factors					
Preterm	0.85	0.03	0.80	0.91	<0.0001
Dopamine	0.71	0.05	0.62	0.81	<0.0001
Total parental nutrition	0.88	0.05	0.78	0.99	0.034
Blood transfusions	0.92	0.05	0.83	1.01	0.110
Malformations	1.17	0.07	1.04	1.31	0.010
Birth Weight (ref NW)					
HBW	1.00	0.04	0.93	1.10	0.947
LBW	1.08	0.02	1.04	1.11	<0.0001
VLBW	1.40	0.21	1.05	1.89	0.023
ELBW	0.99	0.16	0.70	1.34	0.853
Interaction term					
LBW × Preterm	0.89	0.04	0.82	0.96	0.004
VLBW × Preterm	0.65	0.10	0.48	0.89	0.023
ELBW × Preterm	0.97	0.16	0.69	1.34	0.853
Maternal factors					
Maternal autoimmune TD	1.10	0.03	1.01	1.13	0.016
Twin delivery	0.92	0.02	0.89	0.96	<0.0001
Year of birth (2011 ref)					
2012	0.68	0.01	0.67	0.70	<0.0001
2013	0.75	0.01	0.74	0.77	<0.0001
2014	0.95	0.01	0.93	0.97	<0.0001
2015	0.86	0.01	0.84	0.88	<0.0001
2016	0.80	0.01	0.79	0.82	<0.0001
Province of birth (Chieti ref)					
Pescara	0.92	0.01	0.91	0.94	<0.0001
Teramo	0.85	0.01	0.84	0.87	<0.0001
L'Aquila	0.96	0.01	0.94	0.97	<0.0001
Observations 60,817					
R^2 0.36					

Blood transfusions (**Supplementary Figure 2B**), dopamine administration (**Supplementary Figure 2C**), and total parenteral nutrition (**Supplementary Figure 2D**) were associated with significantly lower TSH levels. Interestingly, seasons significantly affected TSH levels, with the peak value observed in winter (**Supplementary Figure 3** and **Supplementary Table 2**). The individual effect of the remaining covariates was less pronounced, although reaching statistical significance (summarized in **Supplementary Figure 3** and **Supplementary Table 2**).

Combined Influence of the Risk Factors on Neonatal TSH Levels

When the covariates were analyzed in a multiple linear regression model that related them to DBS TSH, most of them retained statistical significance after adjusting for the influence of the

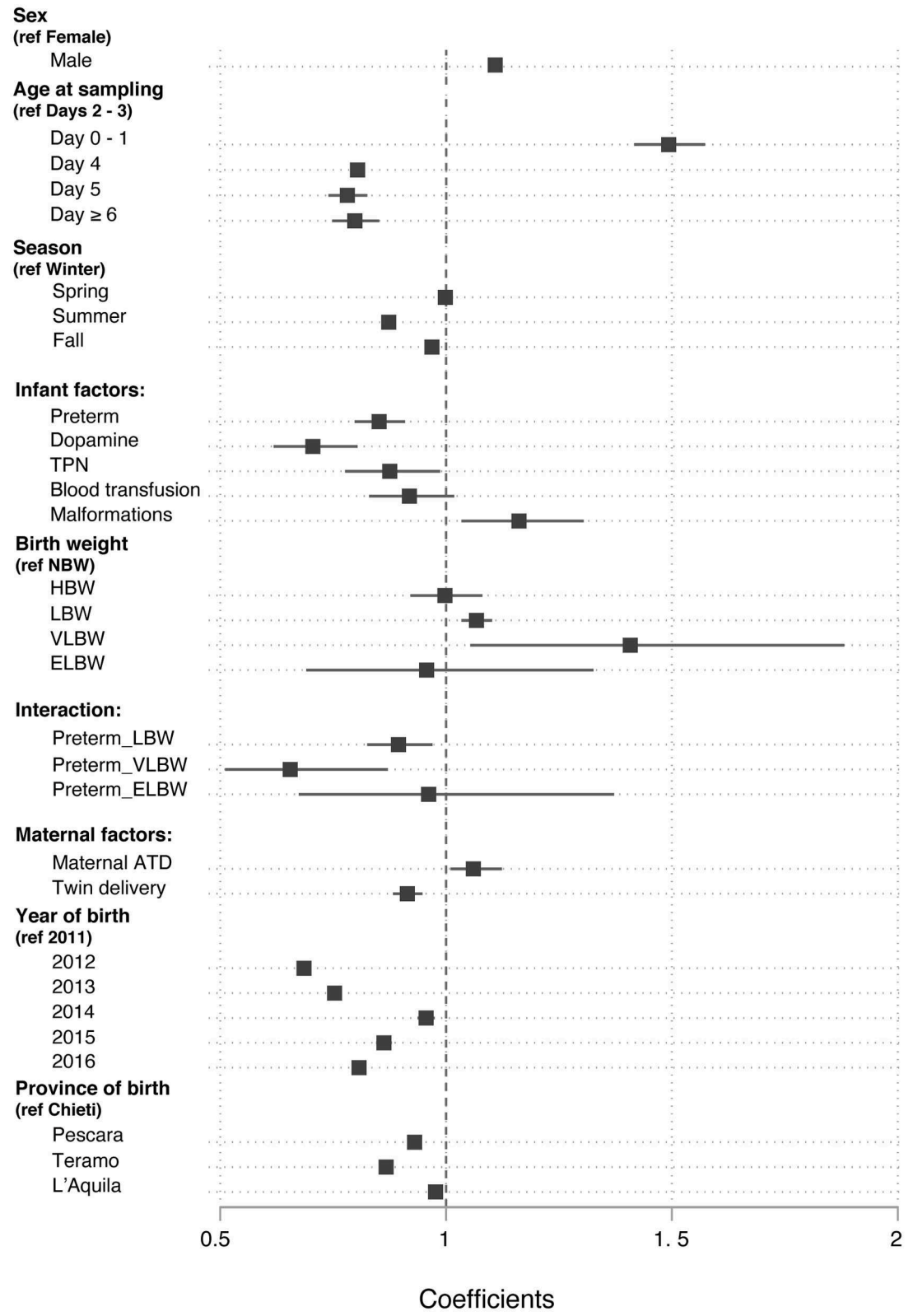

FIGURE 3 | Values of the β regression coefficients in a multiple linear regression model that related the neonatal blood TSH levels to a set of nine covariates.

others (**Table 1** and **Figure 3**). The strength of their association was modest, with regression coefficients ranging from a 50% decrease to 40% increase in TSH levels, none of them causing a 2-fold or greater effect (**Figure 3**).

Infant Factors

Neonatal TSH was influenced by sex: males had higher TSH levels than females (β regression coefficient, β = 1.11; 95% CI 1.09, 1.12; $p < 0.0001$). Neonatal TSH, as expected, was higher on day 0 and 1 compared to days 2–3 (β = 1.49; 95% CI 1.42, 1.57; $p < 0.0001$), whereas it showed a negative correlation with age when measured on day 4, 5, or 6 of life than on days 2–3 (β = 0.78; 95% CI 0.74, 0.83; $p < 0.0001$).

The season of birth also influenced the neonatal TSH levels. Neonates born in summer and autumn had a 13% (β = 0.87; 95% CI 0.86, 0.89; $p < 0.0001$) and 2% (β = 0.98; 95% CI 0.95, 0.98; $p < 0.0001$) lower TSH than those born in winter, respectively.

Preterm had a 15% lower TSH than term infants (β = 0.85; 95% CI 0.80, 0.91; $p < 0.0001$). Moreover, the use of dopamine (β = 0.71; 95% CI 0.62, 0.81; $p < 0.0001$), total parenteral nutrition (β = 0.88; 95% CI 0.78, 0.99; $p = 0.034$), and malformations (β = 1.17; 95% CI 1.04, 1.34; $p = 0.01$) were also significantly associated with TSH. Administration of blood transfusion, on the contrary, had no significant effect on neonatal TSH.

In the univariate analysis, TSH was lower in LBW, VLBW, and ELBW than in NW newborns. However, in the final multiple linear regression model TSH was higher in LBW (β = 1.08; 95% CI 1.04, 1.11; $p < 0.0001$) and in VLBW (β = 1.40; 95% CI 1.05, 1.89; $p = 0.023$) compared to NW newborns, after adjusting for prematurity. The relationship between birthweight and TSH was also influenced by gestational age. Indeed, preterm newborns with LBW (β = 0.89; 95% CI 0.82, 0.96; $p = 0.004$) and VLBW (β = 0.65; 95% CI 0.48, 0.89; $p = 0.023$) had lower TSH levels than term infants.

Maternal Factors

Maternal history of pre-gestational autoimmune thyroid disease was associated with higher neonatal TSH levels in the multiple linear regression analysis (β = 1.10; 95% CI 1.01, 1.13; $p = 0.016$). Twins had an 8% lower TSH value than singletons (β = 0.92; 95% CI 0.89, 0.96; $p < 0.0001$).

Calculation of Neonatal TSH Percentile Charts Based on Sex and Age

The significant effect of sex and age at blood collection had on neonatal TSH levels prompted us to devise percentile charts illustrating the distribution of TSH values in healthy neonates (i.e., at term, normo-weight, not affected by primary CH, **Figure 4**). The charts include eight lines corresponding to the 99, 97.5, 95, 90, 75, 50, 25 and 10th percentile. They depict the slightly elevated TSH values in males during the first two days of life and a more stable trend in the upper quartile of the distribution between the 2nd and 4th day of life (shaded area in **Figure 4**). Local percentile charts may be a useful tool to identify elevated TSH values at each day of postnatal age in each sex.

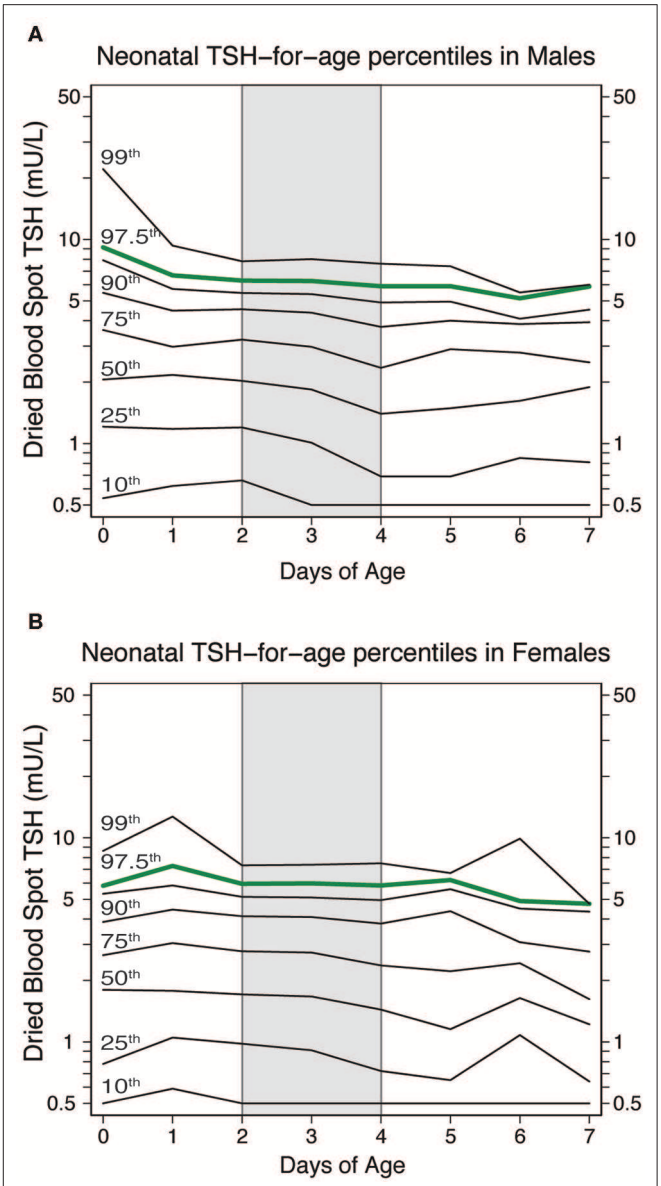

FIGURE 4 | Neonatal TSH percentiles charts in healthy neonates according to their neonatal age and sex [(males in panel **(A)** and females in panel **(B)**]. The green line represents the 97.5th percentiles of neonatal TSH, which could be used to establish TSH cut-off value at the screening centers.

DISCUSSION

We utilized newborn screening data over a 6-year period to examine the associations between TSH levels and several infant and maternal factors. This study revealed that neonatal TSH is influenced by several factors including sex, age at blood collection, prematurity, use of dopamine and total parenteral nutrition, birthweight, season of birth, history of autoimmune maternal thyroid disease, and twin-pregnancy.

Males had significantly higher TSH levels than females in our study population, in keeping with what has been

reported by most studies (9–13). Some studies, however, have failed to show this sex difference (14–17), likely due to smaller sample sizes and/or a focus on preterm newborns. The mechanisms underlying the elevated neonatal TSH levels in males remains unexplained. From a practical perspective, however, this difference should encourage primary CH screening programs to devise their own sex-specific cut-off values. When coupled with age-specific cut-offs, local percentile charts, as we have developed in this study, could improve the accuracy of primary CH screening. The TSH for age and sex percentile charts also underscore the importance of timing the blood collection between the second and fourth day of life (4), so as not to miss cases where TSH is mildly but persistently elevated (18).

The effect of prematurity on neonatal TSH has been extensively investigated. Some studies found lower TSH levels in preterm newborns than in those born after 37 weeks of gestation (10, 19, 20), which is also what we found. Other studies, instead, reported higher TSH values in preterm infants (11, 12, 17), and others no difference in TSH levels according to gestational age (9, 15). Since it has been shown that preterm babies have an immature hypothalamo-pituitary axis (21), it is reasonable to postulate that preterm infants indeed have lower TSH levels and ascribe the variability to differences in blood collection timing and study populations. It has also been proposed that other conditions occurring in preterm, such as drug exposure [dopamine (22), glucocorticoids (23)], total parenteral nutrition (24), sepsis (25), and respiratory distress syndrome (25) decrease TSH values in preterm babies. In our study, the administration of dopamine and total parenteral nutrition was indeed associated with lower TSH levels after controlling for preterm status. However, the lower TSH levels observed in newborns treated with dopamine and TPN may be related to concomitant severe illness, not considered in our multivariate analysis.

The influence of birthweight on TSH remains debatable. Several studies (10–12, 17, 26) have reported higher TSH levels in LBW and VLBW babies than in NW babies. When the effect was analyzed in a multiple regression model, however, we noted it was modified by prematurity. In particular, preterm babies with LBW or VLBW had lower TSH values than those with a normal birthweight, whereas term infants with VLBW had higher TSH values than those with NW. Our findings suggest that preterm infants with LBW or VLBW require repeated TSH testing to properly establish a diagnosis of primary CH, as highly recommended by Hashemipour et al. (6) who systematically reviewed previous works on screening for CH in preterm, LBW, and VLBW infants.

The influence of the season of birth on neonatal TSH has been reported in Belgium (16, 26), Latvia (27), Turkey (28), Iran (29, 30), and Iowa (19), but never in Italy. We found that neonatal TSH is significantly higher in winter than summer and fall, in keeping with what has been reported in Turkey, Iran and Iowa. In Belgium and Latvia, on the contrary, TSH levels were found to be higher during the fall months. The cause(s) of this seasonal variation is not known. A decreased maternal consumption of iodine-rich foods during the winter months could lead to increased TSH levels in the baby. Alternatively, lower environmental temperatures could stimulate the fetal pituitary to produce more TSH. In adults, TSH hypersecretion secondary to low ambient temperatures has indeed been reported in patients with primary hypothyroidism on constant replacement dosage of thyroxine (31). Recently, Yoshihara et al. (32) reported seasonal changes in TSH in 135,417 Japanese adult patients and a negative correlation between TSH and daily temperature. Another possible explanation is the relationship between maternal vitamin D and fetal TSH levels. Barchetta et al. (33) and Das et al. (34) have reported that TSH is higher in winter and inversely correlated with vitamin D levels in euthyroid adults, offering a mechanism to explain TSH seasonality.

Although the history of maternal thyroid disease is generally considered a risk factor for CH (35–37), few studies have actually examined its effect on neonatal TSH. Some authors (15, 16, 25) did not observe a relationship between history of maternal thyroid dysfunction and/or thyroid nodules, and neonatal TSH, either in univariate models or after adjusting for gestational age. Others, focusing on the relation between autoimmune thyroid disease and neonatal TSH levels, reported higher TSH values in neonates born to mothers with autoimmune thyroid disease (38–40). Consistent with the latter studies, we found that newborns born to mothers with pre-gestational history of autoimmune thyroid disease had higher TSH levels in multivariable, but not univariate, models. The trans-placental passage of antibodies blocking the TSH receptor and/or the use of anti-thyroid drugs could be responsible for these neonatal thyroid dysfunctions. However, we did not specifically evaluate the influence of anti-thyroid drugs on neonatal TSH levels.

Twin deliveries represent a challenge for primary CH screening. Our twins had lower TSH than singletons, in keeping with the hypothesis that twins have a reduced post-natal rise in TSH likely due to mixing of fetal blood in monozygotic twins and increased risk of preterm delivery. We consider twins at greater risk of missed or delayed primary CH diagnosis (41–44), and confirm the use of special TSH screening protocols for this category of newborns. The effect of twin pregnancy on TSH, however, is not invariably reported. Ryckman et al. (25) and Bosch-Gimenez et al. (14) reported no effect of twin pregnancy on TSH levels in preterm newborns. In addition, Lee (15) showed that the first of twin babies had higher TSH levels than singletons in univariate analysis. In their multiple regression model, only birth order influenced TSH levels, suggesting that a greater stress occurring during delivery, rather than the fact of being a twin, modified neonatal TSH levels.

Strengths of our study are the comprehensive assessment of the main infant and maternal factors that have been reported to influence neonatal TSH, as well as the large size and unbiased nature of the study population. The multivariate analysis performed is another strength, as it provides the true influence on neonatal TSH of the variables analyzed. Weaknesses are related mainly to the quality of the data source, that is the information recorded on the collection cards. For example, cards listed the gestational age as term or prematurity, rather than a true number, a simplification that limited the possibility to define a neonate small or appropriate for gestational age and thus to better clarify the interaction between birthweight and prematurity.

In addition, our collection cards did not include other factors known to influence neonatal TSH [such as, maternal origin (14), maternal thyroid function during pregnancy (25), iodine supplementation (16), mode of delivery (15), APGAR score (45), and hematocrit (46)], highlighting the need of replicating our findings in more extended datasets. One major limitation is that we lacked measures of iodine status. The Abruzzo region can be considered a mildly iodine deficient area (47). Therefore, we cannot exclude that iodine deficiency, even if mild, may have contributed for any alteration in measured TSH levels in this population.

In conclusion, this study highlights the influence of several infant and maternal factors on neonatal TSH supporting the delineation of own sex- and age-specific TSH cutoff values that can be used to refine local screening protocols. The study also supports the universal adoption of a second TSH screening in neonates at risk of missed primary CH detection.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

GD contributed to the design/plan of the study, performed the statistical analysis, wrote, and revised the manuscript. MC contributed to data collection and revision of the manuscript. DS performed the TSH measurements and contributed to data collection and revision of the manuscript. CG contributed to the study design and revision of the manuscript. GN directs the neonatal screening center and contributed to the study design and revision of the manuscript. PC contributed to the writing and revision of the manuscript. IB co-directs the neonatal screening and contributed to the writing and revision of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2020.00456/full#supplementary-material

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REVIEW
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Neonatal Diabetes Mellitus

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Neonatal Diabetes (ND) mellitus is a rare genetic disease (1 in 90,000 live births). It is defined by the presence of severe hyperglycaemia associated with insufficient or no circulating insulin, occurring mainly before 6 months of age and rarely between 6 months and 1 year. Such hyperglycaemia requires either transient treatment with insulin in about half of cases, or permanent insulin treatment. The disease is explained by two major groups of mechanism: malformation of the pancreas with altered insulin-secreting cells development/survival or abnormal function of the existing pancreatic β cell. The most frequent genetic causes of neonatal diabetes mellitus with abnormal β cell function are abnormalities of the 6q24 locus and mutations of the *ABCC8* or *KCNJ11* genes coding for the potassium channel in the pancreatic β cell. Other genes are associated with pancreas malformation or insufficient β cells development or destruction of β cells. Clinically, compared to patients with an *ABCC8* or *KCNJ11* mutation, patients with a 6q24 abnormality have lower birth weight and height, are younger at diagnosis and remission, and have a higher malformation frequency. Patients with an *ABCC8* or *KCNJ11* mutation have neurological and neuropsychological disorders in all those tested carefully. Up to 86% of patients who go into remission have recurrent diabetes when they reach puberty, with no difference due to the genetic origin. All these results reinforce the importance of prolonged follow-up by a multidisciplinary pediatric team, and later doctors specializing in adult medicine. 90% of the patients with an *ABCC8* or *KCNJ11* mutation as well as those with 6q24 anomalies are amenable to a successful switch from insulin injection to oral sulfonylureas.

Keywords: neonatal diabetes mellitus, chromosome 6q24 abnormality, associated malformations, neuropsychological disorder, KCNJ11 (Kir6.2), ABCC8, sulfonylurea receptor (SUR1)

DEFINITION

Diabetes mellitus in very young children or neonatal diabetes is a rare genetic disease (minimal incidence: 1 in 90,000 live births) with variations within different ethnic groups (1–3). It is defined by the presence of severe hyperglycaemia requiring treatment and occurs between the neonatal period and infancy. It occurs mainly before 6 months of age (155/173 probands in our published cohort) and rarely between 6 months and 1 year (18/173) (4). In the Finnish population for example, after 6 months of age, patients with diabetes had high HLA risk genotypes and islet autoantibodies, reflecting the autoimmune character of diabetes (5). This hyperglycaemia is associated with insufficient or no circulating insulin (3). Two clinical forms have been distinguished, based on the duration of the treatment: a so called "transient form" and a permanent form.

The disease is explained by two major groups of mechanism: malformation of the pancreas or abnormal function of the pancreatic β cell that secretes insulin (by poor insulin cell mass development or malfunction of a cell component or by destruction of the β cell) (**Table 1**) (see **Figure 1** for the normal functioning of the β cell).

GENETIC CAUSES

Abnormal β Cell Function

The most frequent genetic causes of neonatal diabetes with normal pancreas morphology are abnormalities of the 6q24 locus and mutations of the genes coding for the ATP-dependent potassium channel.

6q24 (MIM#601410 and 603044)

The first genetic causes identified were abnormalities of the 6q24 locus, which include paternal uniparental disomy of 6q24 (pUPD6), partial duplication of paternal 6q24 and relaxation of the maternal 6q24 imprinted locus. This locus contains a CpG island, presenting differential methylation depending on the parental origin (non-methylation on the paternal allele, methylation on the maternal allele) (6). To date, the methylation abnormality has not been found in the parents of affected children. Methylation is used to down-regulate gene transcription of the methylated allele. All these abnormalities lead to over-expression of imprinted genes located in 6q24, such as PLAGL1/ZAC (pleiomorphic adenoma gene-like 1) and HYMAI (Hydatidiform mole-associated and imprinted transcript), which are the most "likely" candidate genes (6–8). PLAGL-1 codes for a transcription factor involved in regulation of stopping the cell cycle and apoptosis and in induction of the receptor 1 gene for human pituitary adenylate cyclase-activating polypeptide (PACAP1, which is a potent stimulant of insulin secretion). The function of the HYMAI gene is unknown (9). The mechanism responsible for the diabetes could be linked to a developmental defect in the β cells but the fact that remission of the diabetes occurs means that an abnormality in β cell function cannot be ruled out (10). The 6q24 abnormalities are associated with "transient" neonatal diabetes (7, 8, 11).

The ZFP57 gene (MIM *612192) is involved in maintaining methylation of the DNA during the very early stages of embryogenesis. It is localized at 6p22.1. Homozygous mutations leading to a lack of protein or non-functional protein are associated with widespread DNA hypomethylation, including hypomethylation of the 6q24 locus (12). However, there are patients who have a 6q24 methylation abnormality not due to mutations of this gene (12).

Mutations of the ABCC8 and KCNJ11 Genes Coding for the K_{ATP} Channel: (MIM *600509 and *600937)

The ATP-dependent potassium channel (K_{ATP} channel) plays a central role in stimulating insulin secretion by the pancreatic β cell in response to glucose. At low blood sugar levels (e.g., fasting), the K_{ATP} channels are open (activated) and their activity maintains a hyperpolarized resting membrane potential (around −70 mV). A rise in blood sugar level (e.g., post-prandial) causes increased passage of glucose into the β cell. Glucose enters the glycolysis pathway, which increases the intracellular ATP concentration. This causes the K_{ATP} channels to close (inhibition), which leads to the intracellular potassium accumulation that causes membrane depolarization. This depolarization activates the voltage-dependent calcium channels, leading to Ca^{2+} ions entering the β cell, then enabling exocytosis of the secretion vesicles and release of insulin into the bloodstream (**Figure 1**).

The K_{ATP} channel is an octamer formed from two types of subunits: the Kir6.2 subunits form the channel selective for the incoming corrective potassium enclosed in SUR1 ion-channel regulator subunits (13, 14). They are coded by the *KCNJ11* and *ABCC8* genes, respectively.

Activating mutations in one of these two genes are responsible for neonatal diabetes with normal pancreas morphology (15–17). They result in the K_{ATP} channel remaining permanently open, so that it no longer controls membrane potential in response to glucose and therefore blocks the event cascade that leads to insulin release.

Mutations of the Insulin Gene (INS) (MIM *176730)

The third cause of neonatal diabetes, by frequency, is mutations of the insulin gene (*INS*). The majority are heterozygous mutations affecting the structure of preproinsulin; these are transmitted in an autosomal dominant manner (18, 19). The abnormal proinsulin undergoes degradation in the endoplasmic reticulum, leading to severe endoplasmic reticulum (ER) stress and β cell death. This process has been described in mouse models (20) and in man (21, 22). Recent evidence suggests that *INS* mutations do not necessarily lead to beta-cell death but rather the chronic ER stress interferes with beta-cell growth and development (23).

Some mutations alter expression of the protein. They are transmitted in a recessive manner, in the majority of cases in consanguineous families. These mutations affect the insulin promoter directly of by mutation in factor that enhances its activity (24, 25).

TABLE 1 | Genetic causes of monogenic neonatal diabetes based on physiopathological mechanisms [excluding 6q24 locus abnormalities (MIM *601410, *603044, and *612192)].

Gene/Protein	Function	Locus	Transmission mode	Type of diabetes	Reference OMIM numbers
BETA CELL FUNCTION ABNORMALITY					
ABCC8/SUR1	K_{ATP} channel/insulin secretion	11p15.1	Dominant	PND/TND/iDEND/DEND	MIM *600509
KCNJ11/Kir6.2	K_{ATP} channel/insulin secretion	11p15.1	Dominant	PND/TND/iDEND/DEND	MIM *600937
INS/Insulin	Hormone	11p15.5	Rare Recessive	Isolated TND/PND	MIM *176730
GCK/Glucokinase	Glucose metabolism	7p15.3-p15.1	Recessive/Dominant	Heterozygous: MODY2 Homozygous: PND	MIM *138079
SLC2A2/GLUT2	Membrane receptor	3q26.1-q26.2	Recessive	PND/TND + Fanconi-Bickel syndrome (glycogenosis) Proximal tubulopathy + small size + rickets + abnormality of glucose and galactose metabolism	MIM *138160
SLC19A2	Thiamine transporter	1q23.3	Recessive	Rogers Syndrome: Thiamine-sensitive megaloblastic anemia + diabetes + perceptive deafness ± PND	MIM *603941
ENDOCRINE PANCREAS DEVELOPMENT ABNORMALITY					
GATA6/GATA6	Transcription factor	18q11.1-q11.2	Dominant	PND by pancreas agenesis/hypoplasia + congenital cardiopathy + biliary tract abnormalities	MIM *601656
GLIS3/Zinc finger protein, GLIS3	Transcription factor	9p24.2	Recessive	PND + congenital hypothyroidism ± progressive hepatic fibrosis ± cystic renal dysplasia ± congenital glaucoma	MIM *610192
HNF1β/HNF1β	Transcription factor	17q12	Dominant	MODY5 or TND + pancreatic hypoplasia + renal cyst	MIM *189907
NEUROD1/BETA2	Transcription factor	2q31.3	Recessive/Dominant	Heterozygous: MODY6 Homozygous: PND + cerebellar hypoplasia + visual defect + perceptive deafness	MIM *601724
NEUROG3/Neurogenin3	Transcription factor	10q21.3	Recessive	Homozygous hypomorphic mutation: congenital malabsorption diarrhea + late-onset diabetes (8 years) Homozygous nonsense mutation: PND + congenital malabsorption diarrhea	MIM *604882
PAX6/aniridia type II protein, Pax6	Transcription factor	11p13	Recessive	PND + microphthalmia + cerebral malformation	MIM *607108
PDX1 (or IPF1)/ Pancreas/duodenum homeobox protein 1	Transcription factor	13q12.1	Recessive/Dominant	Heterozygous: MODY4 Homozygous nonsense mutation: PND by agenesis/hypoplasia of the pancreas Homozygous hypomorphic mutation: PND by hypoplasia of the pancreas	MIM *600733
PTF1A/Pancreas Transcription Factor 1	Transcription factor	10p12.2	Recessive	PND by agenesis of the pancreas + cerebellar agenesis	MIM *607194
RFX6/Rfx6	Transcription factor	6q22.1	Recessive	Martinez-Frias Syndrome: Pancreatic hypoplasia + intestinal atresia with diarrhea + agenesis/hypoplasia of the gall bladder	MIM *612659
CNOT1	Transcriptional repressor	16q21	*De novo* specific mechanism of the mutation	Pancreatic agenesis + holoprosencephaly	MIM *604917

(Continued)

TABLE 1 | Continued

Gene/Protein	Function	Locus	Transmission mode	Type of diabetes	Reference OMIM numbers
DESTRUCTION/ENDOPLASMIC RETICULUM STRESS WITH LOW INSULIN CELL MASS OR DESTRUCTION OR EARLY IMMUNE DESTRUCTION OF THE BETA CELLS					
INS/Insulin	Hormone	11p15.5	Dominant	PND	MIM *176730
EIF2AK3/EIF2AK3	Enzyme	2p11.2	Recessive	Wolcott Rallison Syndrome: PND + epiphyseal dysplasia	MIM *604032
IER3IP1/immediate early response 3-interacting protein 1	Endoplasmic reticulum protein	18q12	Recessive	PND + microcephaly + lissencephaly + epilepsy	MIM *300292
FOXP3/Forkhead box protein P3	Transcription factor (Forkhead domain)	Xp11.23	X-linked recessive	IPEX syndrome: Immunodysregulation Polyendocrinopathy Enteropathy X-linked: PND + increased IgE levels	MIM *300292
STAT3	Transcription factor	17q21.2	Dominant	Autoimmune disease, multisystem PND	MIM *102582
WFS1/Wolframin	Transmembrane protein of the endoplasmic reticulum	4p16.1	Recessive	Wolfram Syndrome: PND + optic atrophy ± diabetes insipidus ± deafness (DIDMOAD)	MIM *222300

ND, neonatal diabetes; PND, permanent neonatal diabetes; TND, transient neonatal diabetes; DEND, Developmental delay, Epilepsy and Neonatal Diabetes; iDEND, intermediate DEND = DEND without epilepsy; MODY, Maturity Onset Diabetes of the Young.

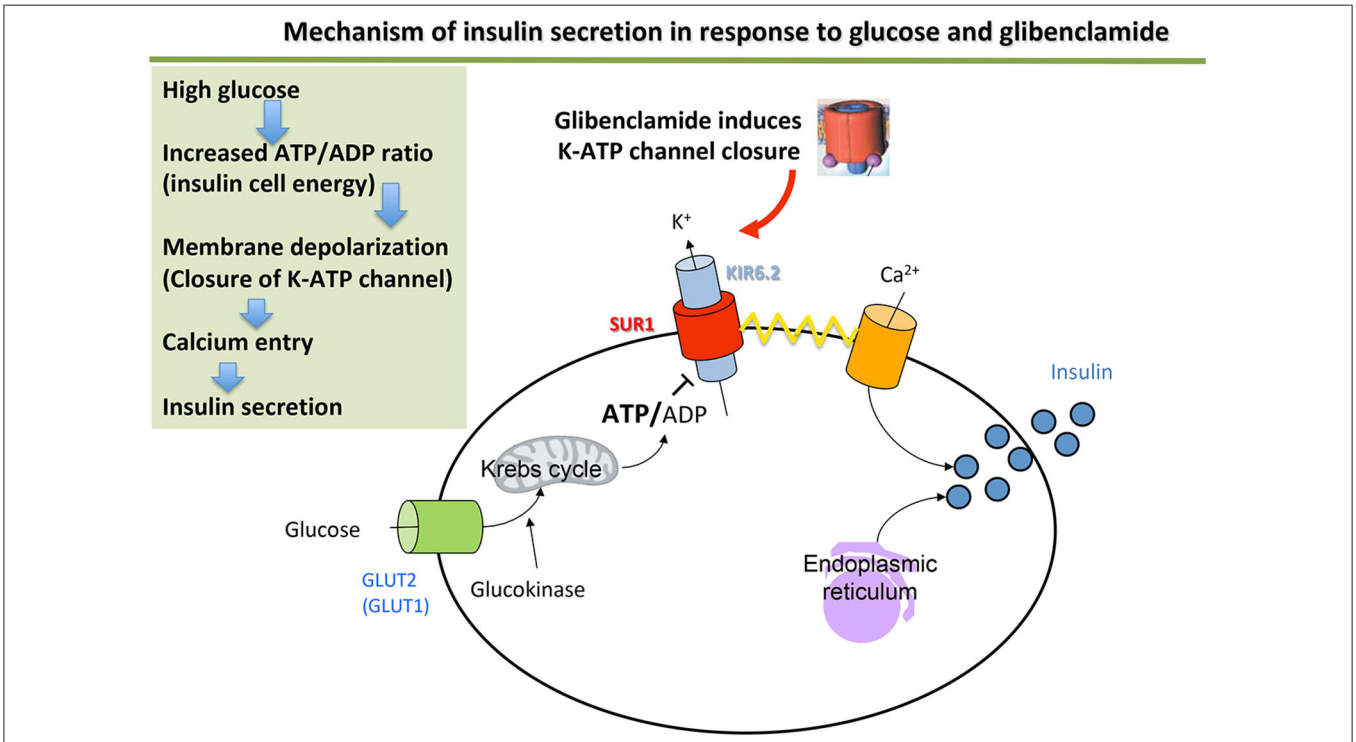

FIGURE 1 | Mechanism of insulin secretion in response to glucose and glibenclamide.

Mutations of the Glucokinase Gene (MIM *138079)

Glucokinase is responsible for the first step of glucose metabolism in the β cell. It acts as a "sensor" of blood glucose, making it possible to control the quantity of insulin secreted. Nonsense mutations of the glucokinase gene cause MODY 2 (Maturity onset diabetes in the youth type 2), which usually presents as moderate hyperglycaemia (26). Transmission is heterozygous. In the homozygous state, these nonsense mutations cause neonatal diabetes by complete deficiency of glucokinase-mediated glycolysis (27). This is not a frequent cause of neonatal

diabetes (28, 29). However, an assay of the fasting blood glucose concentration is required from both parents, particularly if there is a history of gestational diabetes. The discovery of discreet glucose intolerance in both parents should therefore lead to a search for glucokinase gene mutations.

Abnormal Pancreas Morphology

Several genes are linked to neonatal diabetes with abnormal pancreas morphology and precise description is beyond the scope of this chapter (see **Table 1** for a brief information). These genes are involved in development of the pancreas at various stages in early morphogenesis. These neonatal diabetes cases may be associated with a deficiency of the exocrine pancreas, based on the severity of pancreatic damage or to other congenital malformations. Mutation of the RFX-6 gene deserves a specific comment. The RFX-6 transcription factor is involved in the differentiation of beta-cells in the pancreas during embryonic development of the pancreas. It is also expressed in mature cells where it has a role in regulating insulin transcription and secretion. It actually controls the expression and activation of calcium channels and its inactivation alters insulin secretion in response to glucose. A few cases of neonatal diabetes have been reported. Patients display developmental abnormalities of the pancreas and of the digestive tract. The mechanism is linked to both a developmental and a functional disorder of the endocrine pancreas. Transmission is autosomal recessive (**Table 1**).

Autoimmune Neonatal Diabetes Mellitus

Most patients diagnosed with diabetes between 6 and 12 months of age will have the "typical" type 1 diabetes mellitus seen in older children with positive autoantibodies against the beta cell. Autoimmune diabetes is very rare before 6 months of age and will most often be linked to specific causes.

IPEX Syndrome (Table 1)

Mutations of the FOXP3 gene may be responsible for enteropathy, immune dysregulation and polyendocrinopathy. It is a cause of neonatal diabetes associated with early autoimmunity directed against the beta cells of the pancreas. This diagnosis should be considered in male infants presenting diabetes associated with immune deficiency and/or severe infections. Immunosuppressant treatment can be considered (serolimus, corticosteroids) but bone marrow transplant must be considered as soon as the child's clinical condition allows. Insulin treatment will be combined with specialized nutritional management (parenteral ± enteral nutrition) before and after the transplant. It should be noted that, while correcting immune deficiencies, this will not eliminate the diabetes.

Down Syndrome and Neonatal Diabetes

Patients with Down syndrome (DS) resulting from trisomy 21 are more likely to have childhood diabetes mellitus. Professor Hattersley's group found 13 infants affected by DS who were diagnosed with diabetes before the age of 6 months. Trisomy 21 was seven times more likely in their PNDM cohort than in the general population (13 of 1,522 = 85 of 10,000 observed vs. 12.6 of 10,000 expected). Known PNDM genes explains 82.9% of non-DS PNDM in their work. None of the 13 DS-PNDM patients had a mutation in those genes. The conclusion from this work is that trisomy 21 is a cause of autoimmune PNDM that is not HLA associated (30).

Other mutations, such as the activating STAT3 mutations have been described which cause neonatal diabetes associated with beta-cell autoimmunity (**Table 1**).

CLINICAL DESCRIPTION

There are two clinical forms of neonatal diabetes based on the duration of insulin-dependency. In the transient form, treatment may be stopped at any time from the first weeks of life to 5 years of age (4). In the permanent forms, life-long treatment is necessary.

The clinical difference between transient and permanent neonatal diabetes is not always underpinned by distinct molecular mechanisms. Abnormalities of the 6q24 locus are exclusively linked to transient neonatal diabetes. However, mutations of the *ABCC8*, *KCNJ11*, and *INS* genes are linked to both permanent and transient forms (17, 18, 25). Other genetic causes are associated with permanent neonatal diabetes.

Neonatal diabetes is usually diagnosed before 6 months of age. However, the age of diagnosis varies depending on genetic causes: diabetes due to a 6q24 locus abnormality appears before the age of 1 month in 93% of cases and before the age of 3 months in 100% of cases. In *ABCC8* and *KCNJ11* gene mutations, it appears before the age of 1 month in 30% of cases and between 1 and 6 months in 66% of cases (4).

At birth, patients have a birth-weight below the 10th percentile in 62% of cases (4), highlighting the crucial role of insulin secretion in fetal growth. This intrauterine growth retardation is found in all genetic groups with a greater proportion in patients with a 6q24 abnormality than those carrying a *ABCC8* or *KCNJ11* mutation (92 vs. 48%, $p < 0.001$) (4).

Half of patients with a detectable pancreas by ultrasound experience remission from the diabetes in our cohort (4). This occurs at the age of about 4 months. There is a difference depending on the genetic cause. Patients with a 6q24 locus abnormality are in remission before the age of 1 year in 97% of cases (median age 14 weeks) while remission may go as far as the age of 5 years in patients with an *ABCC8* or *KCNJ11* mutation (median age 39 weeks) (4, 31). Patients with a rare recessive mutation of the *INS* gene have remission at a median age of 12 weeks (24), whereas the majority of the *INS* gene mutations are dominant and they never go into remission. The diabetes frequently relapses (in up to 86% of cases) at the onset of puberty, probably due to the insulin resistance of puberty (4, 32). There is no difference between the genetic groups.

Depending on the genetic cause, patients with neonatal diabetes may have other clinical signs associated with diabetes (**Table 1**).

In neonatal diabetes with normal pancreas morphology, there are associated neurological disorders and developmental defects. Approximately 25% of patients with a mutation of the *ABCC8* or *KCNJ11* genes have neurological disorders

ranging from psychomotor disorders to delayed cognitive development associated with severe epilepsy (DEND syndrome: Developmental delay, Epilepsy, and Neonatal Diabetes) (33). In addition, we have shown that when patients undergo detailed neuro-psychomotor and neuropsychological tests, an attention deficit or language disorder extending as far as dyslexia is found in 100% of cases (4).

Patients with a 6q24 locus abnormality may have developmental defects (macroglossia, umbilical hernia, cardiac malformations, renal and urinary malformations, non-autoimmune anemia, hypothyroidism with gland *in situ*) and neurological disorders (4, 11).

In neonatal diabetes with abnormal pancreas morphology or with β cell destruction, the associated malformations depend on the genetic causes and are often grouped into defined syndromes (**Table 1**). **Figure 2** illustrates a diagnostic strategy by molecular biology.

Recent long-term follow-up data in TNDM support a decrease in maximal insulin secretion capacity to both glucose and arginine stimuli that reflect low insulin mass (34). This study also showed that, regardless of the underlying genetic abnormalities or the duration of diabetes, TNDM was associated with learning difficulties at school. The high relapse rate and absence of identified predictors of relapse in TNDM suggest a need for an HbA1c assay at least every 2 years throughout childhood and for an HbA1c assay and oral glucose tolerance test every year throughout adolescence (34). During childhood, close attention should be directed to education and neurodevelopmental milestones, in TNDM patients with and without diabetes (34).

THERAPEUTIC ASPECTS

Drug Treatment

Due to the early onset and associated delayed intrauterine retardation, patients with neonatal diabetes very often receive their initial treatment in a neonatal department. The initial treatment aims to rebalance carbohydrate metabolism. It should be started immediately following diagnosis. The treatment consists of the balance between a calorie and carbohydrate intake necessary to restore normal weight without being excessive to avoid the risk of future insulin resistance (15–18 g/kg/d carbohydrate) and sufficient insulin-based treatment to achieve the correct metabolic equilibrium. Restricting intake below the nutritional recommendations for children with low birth weight is ineffective given the physiopathology of circulating insulin deficiency.

Insulin-based treatment is difficult to manage due to the very low weight. The therapeutic margins between hypoglycemia and hyperglycemia are small, and both are harmful for neurological development of the newborn. Using an insulin pump with or without dilution of the insulin to 1:10 in 0.9% NaCl (or with a bona-fide diluent if available) can sometimes improve manageability of the insulin during the first weeks of life (35, 36). Blood glucose meters must be able to give a reliable measurement of capillary blood sugar level with the smallest possible quantity of blood (e.g., 0.3 μl blood). Few "conventional" blood glucose meters meet this criterion. Conventional capillary measurements can be done on the side edge of all the fingers, using auto-lancets offering variable pricking depths. This offers the advantage of sparing newborns' heels. An alternative is to use continuous glucose sensors, either isolated or combined with an insulin pump. In addition to enabling rapid access to interstitial blood glucose (they provide a proxy but do not actually measure the blood glucose value), they can now be coupled to the insulin pump, making it possible to activate the system to stop the insulin pump during hypoglycemia or before it occurs. They also have the advantage of minimizing the number of pricks of the skin. Used under suitable hygiene conditions, there is no increase in skin infections. It is advisable to involve experienced clinicians when treating the child and using these techniques.

Patients with *ABCC8* or *KCNJ11* mutations are treated successfully using hypoglycemic sulfonylureas, which act by binding to the regulator SUR1 subunit of the potassium channel (37) (**Figure 1**). The mutated channels remain sensitive to sulfonylureas in 90% of cases, having an inhibitory effect on the potassium channel of the pancreatic β cell and restoring insulin secretion in response to a meal (38). Sulfonylurea therapy appears to be safe and often successful in neonatal diabetes patients before genetic testing results are available (39). An empiric inpatient trial of sulfonylurea can be therefore considered (39). However, obtaining a genetic diagnosis remains imperative to inform long-term management and prognosis.

It has now been demonstrated that treatment with Sulfonylureas provide a better metabolic equilibrium than insulin by normalizing the HbA1c while strongly reducing the incidence of hypoglycemia in cases of neonatal diabetes with *ABCC8* or *KCNJ11* mutations. It was also shown recently that hypoglycemic sulphonylureas were able to improve neurological, neuropsychological and visuomotor impairment if they are introduced early in the child's life (33, 40, 41). Finally, a recent study has shown that it could sometimes be used successfully to replace insulin in neonatal diabetes associated with chromosome 6 methylation abnormalities (42). This emphasizes the importance of making a genetic diagnosis rapidly after diagnosing neonatal diabetes, and especially the early introduction of sulphonylureas. The clinician's aim will be to treat the child with the maximum dose that normalizes blood glucose levels (pre-prandial target: 70–120 mg/dL—post-prandial target: 100–145 mg/dL) without causing hypoglycemia, in order to optimize the drug's effect on the central nervous system. Sulphonylureas are currently only available as a 5 mg tablet and are not licensed for indications in neonatal diabetes. However, glibenclamide has recently obtained the orphan-drug indication from the European Medicine Agency (EMA) in neonatal diabetes. Unlicensed administration is currently achieved by parents through crushing and extemporaneous dilution of the tablets. However, the crushed tablets are poorly soluble in water, which may lead to variations in the dosage actually received by the child. To resolve this problem, a sulphonylurea suspension called Amglidia^{R} has demonstratable efficacy in this indication (43) and has recently obtained a European Marketing Authorization; it has been available in France under a temporary authorization for use (ATU: Autorisation Temporaire

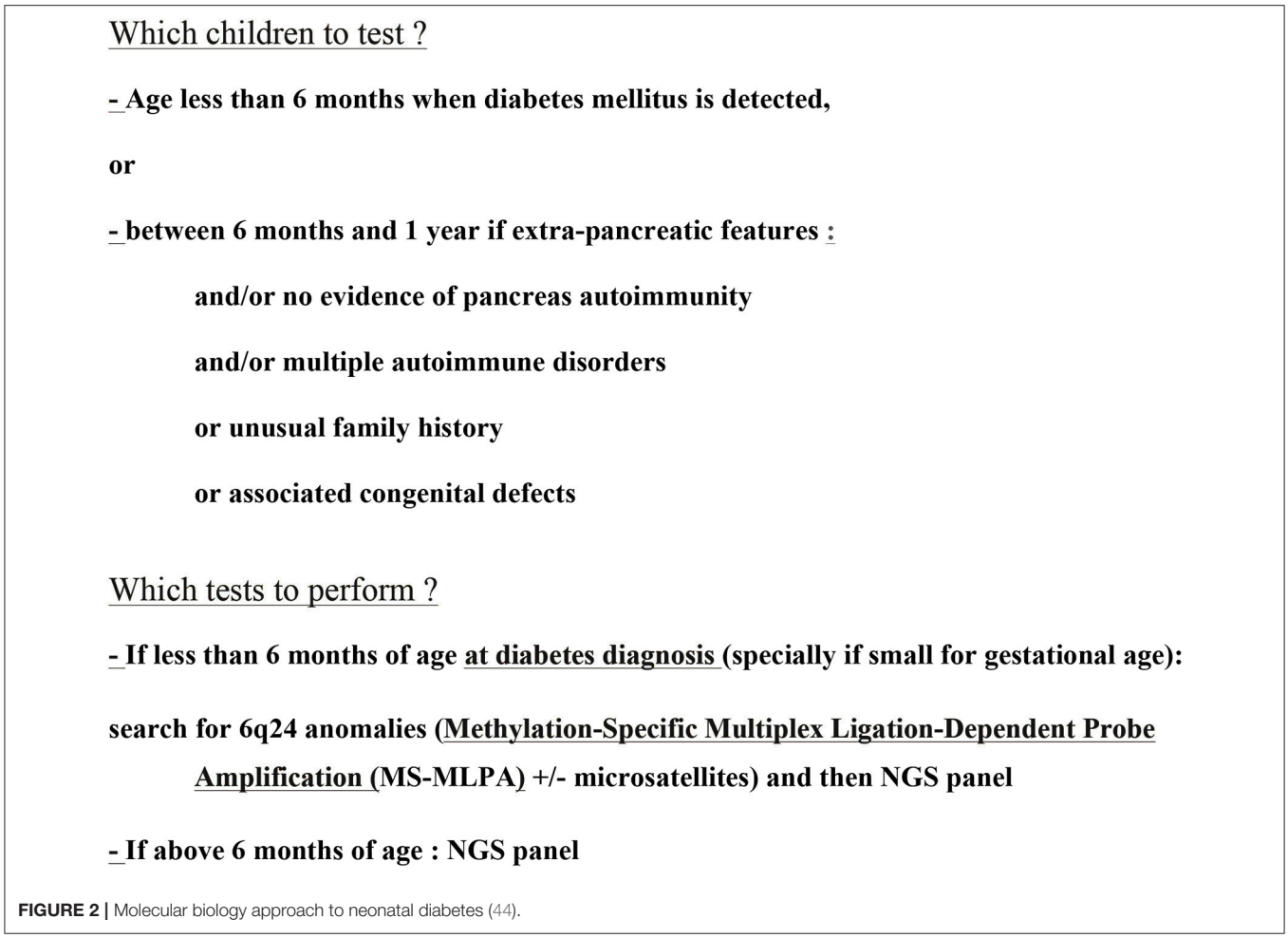

FIGURE 2 | Molecular biology approach to neonatal diabetes (44).

d'Utilization) since 2019. It will enable dosages to be adapted more accurately.

An **Appendix** added to this text describes succinctly the practical aspects of the switch from insulin injection to the glibenclamide suspension licensed in European Union for children and refers to the official summary of product characteristics for detailed information.

Importance of the Genetic Diagnosis

Genetic analyses enables the diagnosis of monogenic diabetes in nearly 83% of diabetes diagnosed before the age of 6 months (30). This genetic diagnosis is essential as it will both influence the therapeutic treatment and make it possible to predict potential diabetes-related complications or illnesses. Genetic analyses must be carried out when diagnosing diabetes mellitus in all of the following children: age <6 months when diabetes mellitus is detected, or between 6 months and 1 year if extra-pancreatic features and/or no evidence of pancreas autoimmunity and/or multiple autoimmune disorders or unusual family history or associated congenital defects (**Figure 2**) (44). Testing should not be delayed until other symptoms of the disease appear or potential remission of the disease. It is also of utmost importance to identify if the sulfonylureas can be introduced successfully as high-dose sulfonylurea therapy has been shown to be an appropriate treatment for patients with KCNJ11 permanent neonatal diabetes from diagnosis. This therapy has been shown to be safe and highly effective, maintaining excellent glycemic control for at least 10 years (45).

CONCLUSION

Neonatal diabetes is a model of rare human genetic disease, important in the understanding of the development and function of the pancreatic β cell, and in helping to resolve the pathophysiology of more frequent adult diabetes, such as type 2 diabetes. Neonatal diabetes is often associated with specific neuropsychological or developmental disorders of underlying genetic causes. A multidisciplinary approach is therefore essential. All clinicians called upon to treat a patient with neonatal diabetes should look for these clinical signs. Knowing the natural history and complete phenotype of this disease makes it possible, firstly, to offer patients better treatment and, secondly, to broaden the scope of genetic analyses to genes involved in the development and function of other organs. Long-term follow-up should be implemented, including for the so-called "transient" forms of neonatal diabetes.

AUTHOR CONTRIBUTIONS

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

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2020.540718/full#supplementary-material

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Current Insights Into Adrenal Insufficiency in the Newborn and Young Infant

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Adrenal insufficiency (AI) is a potentially life-threatening condition that can be difficult to diagnose, especially if it is not considered as a potential cause of a child's clinical presentation or unexpected deterioration. Children who present with AI in early life can have signs of glucocorticoid deficiency (hyperpigmentation, hypoglycemia, prolonged jaundice, poor weight gain), mineralocorticoid deficiency (hypotension, salt loss, collapse), adrenal androgen excess (atypical genitalia), or associated features linked to a specific underlying condition. Here, we provide an overview of causes of childhood AI, with a focus on genetic conditions that present in the first few months of life. Reaching a specific diagnosis can have lifelong implications for focusing management in an individual, and for counseling the family about inheritance and the risk of recurrence.

Keywords: adrenal insufficiency, Addison's disease, adrenal hypoplasia, congenital adrenal hyperplasia, glucocorticoid, DAX-1, MIRAGE syndrome, genetic testing

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INTRODUCTION

Adrenal insufficiency (AI) is a potentially life-threatening condition that needs urgent diagnosis and treatment (1–4). AI is relatively rare in early life, affecting approximately 1:5,000–10,000 children, and its features can be non-specific. Children can be initially mis-diagnosed as having sepsis, metabolic conditions, or cardiovascular disease, highlighting the need to consider adrenal dysfunction as a differential diagnosis for an unwell or deteriorating infant. Prompt recognition allows the correct investigations to be undertaken urgently and definitive management to be established.

AI can be broadly divided into *secondary* causes, due to disruption of hypothalamic or pituitary (corticotrope) ACTH release, and *primary* causes, which affect the adrenal gland itself. Although some conditions have fairly typical presentation patterns and ages of onset, there is often a spectrum of features, and milder variants may produce partial or delayed onset forms of classic conditions (5, 6). Associated features can sometimes give a clue to the diagnosis.

Here, we provide a brief summary of the genetic causes of AI that tend to present in the neonatal period or first few months of life, and the implications of making a specific genetic diagnosis for management. While the focus of this minireview is very much on genetic causes, physical causes (such as adrenal hemorrhage or infiltration) should not be overlooked.

SECONDARY ADRENAL INSUFFICIENCY

Secondary AI is caused by impaired ACTH synthesis and release from pituitary corticotrope cells (**Figure 1A**). ACTH deficiency can be isolated or can occur as part of a combined (multiple) pituitary hormone deficiency (CPHD) due to defects in hypothalamo-pituitary function (**Table 1**). Usually glucocorticoid release is affected, whereas disturbances in mineralocorticoid function and salt balance are unusual as aldosterone synthesis is primarily under the control of the renin-angiotensin system.

Combined Pituitary Hormone Deficiency

Several genetic causes of CPHD are reported (e.g., *GLI1*, *HESX1*, *LHX3*, *LHX4*, *SOX3*, *SOX2* and others) (8). Pituitary ACTH insufficiency usually occurs together with loss of other anterior pituitary hormones (GH, TSH, LH/FSH). Concomitant GH and ACTH insufficiency often causes hypoglycemia in young children, and a small penis and undescended testes may be a sign of congenital gonadotropin insufficiency in boys (9). Other associated features include septo-optic dysplasia or specific associations such as micro-ophthalmia (*SOX2*, *OTX2*). Disruption of *PROP1* or *GH1* can cause ACTH insufficiency in later life (10).

Isolated ACTH Deficiency

Isolated ACTH deficiency can occur due to disruption of TPIT (*TBX19*), or with associated features due to defects in pro-opiomelanocortin (*POMC*) or pro-hormone convertase-1 (PC-1/*PCSK1*).

TPIT is a transcription factor that regulates synthesis of POMC in pituitary corticotrope cells, but not in other POMC producing cells of the body (e.g., skin, hypothalamus) (11). POMC is a precursor molecule that is cleaved to release ACTH along with other peptides (e.g., alpha-MSH, beta-endorphin) (**Figure 1A**). Children with severe disruption of TPIT usually present with evidence of glucocorticoid insufficiency, such as hypoglycemia or hypoglycemic seizures, and prolonged conjugated hyperbilirubinemia in the first few weeks of life (12, 13). This contrasts to *late-onset* isolated ACTH insufficiency, where the molecular basis is currently unknown.

Defects in POMC itself also result in ACTH insufficiency and adrenal dysfunction in early infancy (14). Children have red (or auburn) hair and pale skin due to MSH deficiency, and profound hyperphagia and weight gain from later infancy due to hypothalamic POMC disruption (15). MC4R agonists, which mimic MSH, have had promising results in suppressing hyperphagia in this condition, so it is an important diagnosis to make (16).

Disruption of the cleavage enzyme prohormone convertase-1 (PC-1, *PCSK1*) also presents with ACTH insufficiency, together with hypoglycemia, malabsorptive diarrhea, obesity, and hypogonadism (17, 18). This diagnosis is rare.

PRIMARY ADRENAL INSUFFICIENCY

An overview of monogenic causes of primary adrenal insufficiency (PAI) in childhood is shown in **Table 1** and **Figures 1A,B**, together with inheritance patterns and associated features. Here, we focus primarily on key genetic causes of PAI that present in the first few months of life. Disorders of salt-balance (e.g., aldosterone synthase deficiency) are not included.

Disorders of Steroidogenesis

Smith–Lemli–Opitz Syndrome

Smith–Lemli–Opitz syndrome is a defect in cholesterol biosynthesis due to disruption of the enzyme 7-dehydrocholesterol reductase (*DHCR7*) (19). Common findings in infancy are microcephaly, cleft palate, syndactyly of the second and third toes, post-axial polydactyly, congenital heart defects, gastrointestinal issues (e.g., pyloric stenosis), atypical genital and undescended testes (46,XY) and characteristic facial features (20). AI or impaired stress response can occur, but are surprisingly rare (21, 22). Elevated 7-dehydrocholesterol is diagnostic, coupled with genetic testing.

Early Steroidogenic Defects (*STAR/CYP11A1*)

Steroidogenic acute regulatory protein (*STAR*) plays a key role in cholesterol transport into the mitochondria, whereas the P450 cholesterol side-change cleave enzyme (P450scc, encoded by *CYP11A1*) catalyzes the conversion of cholesterol to pregnenolone (**Figure 1**) (23, 24). These proteins are both required for adrenal (glucocorticoid, mineralocorticoid) and gonadal (testosterone, estrogen) steroid synthesis. Severe disruption causes salt-losing AI in all children and female-typical external genitalia in 46,XY infants. The onset of PAI usually occurs at around 3–4 weeks of age in complete STAR deficiency (also known as "congenital lipoid adrenal hyperplasia"), as build-up of intracellular cholesterol takes time to cause cellular damage ("two-hit hypothesis") (25). In contrast, infants with a severe P450scc deficiency usually present with salt-losing PAI at around 7-10 days (26, 27). Partial disruption of these proteins results in predominant glucocorticoid insufficiency in childhood (27–31).

Congenital Adrenal Hyperplasia

The most common cause of PAI in the first month of life is congenital adrenal hyperplasia (CAH) (23). In virtually all populations, 21-hydroxylase deficiency (21-OHD, *CYP21A2*) is most prevalent, with an incidence of 1:10,000–1:20,000 (although geographical hotspots occur) (32, 33). Other rare forms of CAH include 11 beta-hydroxylase deficiency (*CYP11B1*) (especially in Jewish populations originating from Morocco), 3 beta-hydroxysteroid dehydrogenase deficiency (*HSD3B2*), 17 alpha-hydroxylase deficiency (*CYP17A1*) and P450 oxidoreductase deficiency (*POR*), all of which have specific presentations and biochemical profiles (**Table 1**) (23).

Approximately 80% of 46,XX girls with confirmed 21-OHD have atypical genitalia at birth, so any baby with genital differences and non-palpable gonads should be considered as having 21-OHD until proven otherwise (33, 34). Progressive salt

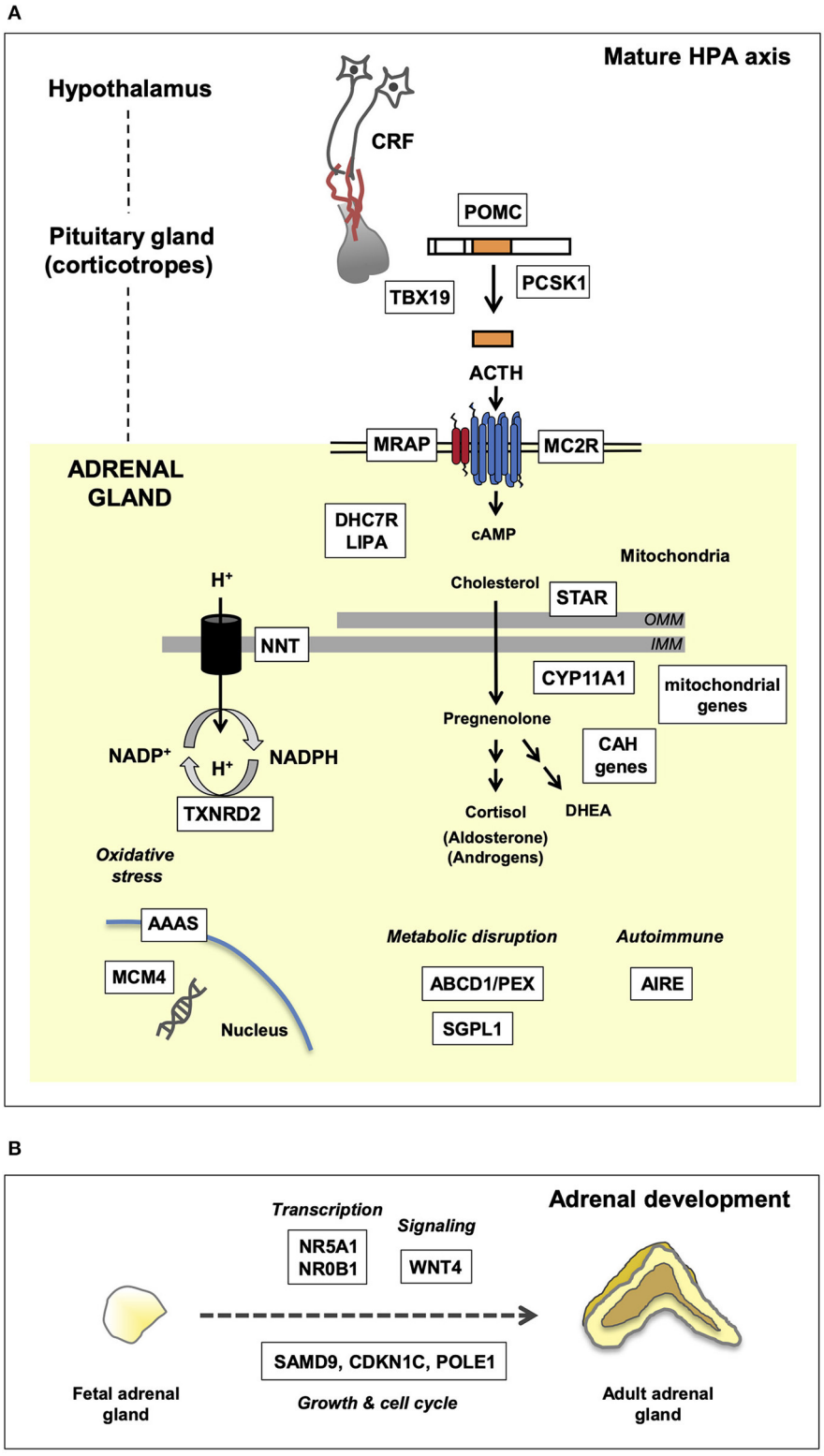

FIGURE 1 | Genetic mechanisms of pediatric adrenal insufficiency (AI) along the hypothalamo-pituitary-adrenal (HPA) axis. Key genes are shown in white boxes. **(A)** Genetic causes of AI as they relate to the mature HPA axis. These genes are required for a multitude of key enzymatic and biochemical processes occurring within the nucleus, mitochondria, and cytoplasm. Disruption of these genes gives rise to the clinical phenotypes discussed in the text. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane. **(B)** Overview of adrenal development and key genes associated with adrenal hypoplasia. Adrenal hypoplasia is mediated by disruption of key genes required for normal fetal adrenal development. These genes are involved in transcription, signaling, and growth/cell cycle processes.

TABLE 1 | Selected monogenic causes of adrenal insufficiency in children.

Condition	Gene (PROTEIN if different)	Inheritance	Associated features
Secondary adrenal insufficiency			
CPHD	*GLI1*, *HESX1*, *LHX3*, *LHX4*, *SOX3*, *SOX2*, *OTX2* and others; also *PROP1*, *GH1* (delayed)	Variable	Many variable associated features including holoprosencephaly, tooth abnormalities (*GLI1*); septo-optic dysplasia (esp. *HESX1*); short rigid neck, hearing loss (*LHX3*); anophthalmia (*SOX2*, *OTX2*)
Isolated ACTH deficiency	*TBX19* (TPIT)	AR	
POMC deficiency	*POMC* (Pro-opio-melanocortin)	AR	Obesity, red hair
Prohormone convertase deficiency	*PCSK1* (PC-1)	AR	Obesity, hypoglycemia, hypogonadotropic hypogonadism
Primary adrenal insufficiency			
Disorders of steroidogenesis			
Smith–Lemli–Opitz syndrome	*DHC7R* (7-dehydrocholesterol reductase)	AR	Syndactyly, polydactyly, facial features, microcephaly, cardiac defects, gastrointestinal features, hypospadias/undescended testes
Congenital lipoid adrenal hyperplasia[a]	*STAR*	AR	46,XY DSD, impaired gonadal steroidogenesis
P450 side chain cleavage def.[a]	*CYP11A1* (P450scc)	AR	46,XY DSD, impaired gonadal steroidogenesis
21-hydroxylase def. (CAH)	*CYP21A2* (P450c21)	AR	46,XX DSD, virilization, early puberty
11β-hydroxylase def. (CAH)	*CYP11B1* (P450c11)	AR	46,XX DSD, virilization, early puberty, hypertension
3β-hydroxysteroid dehydrogenase type 2 def. (CAH)	*HSD3B2* (3β-HSD2)	AR	46,XY DSD, impaired gonadal steroidogenesis; 46,XX DSD, clitoromegaly
17α-hydroxylase/17,20-lyase def. (CAH)	*CYP17A1* (P450c17)	AR	46,XY DSD, impaired gonadal steroidogenesis, hypertension
P450 oxidoreductase def. (CAH)	*POR* (P450 oxidoreductase)	AR	Antley-Bixler syndrome (craniosynostosis, skeletal features, choanal atresia), atypical genitalia (46,XY and 46,XX), impaired gonadal steroidogenesis at puberty
Adrenal hypoplasia			
X-linked AHC	*NR0B1* (DAX-1)	X-linked	Hypogonadotropic hypogonadism, impaired spermatogenesis
Steroidogenic factor-1	*NR5A1* (SF-1)	AD, AR, SLD	46,XY DSD, asplenia
IMAGe syndrome	*CDKN1C*	Imprinted	IUGR, metaphyseal dysplasia, genital anomalies
IMAGe-like syndrome with immunodeficiency	*POLE1*	AR	IUGR, skeletal changes, adrenal hypoplasia, genital anomalies, infections/immunodeficiency, developmental dysplasia of the hip, post-natal growth restriction/facial features
MIRAGE syndrome	*SAMD9*	AD (*de novo*)	Infections, IUGR/preterm, gonadal dysfunction, enteropathy, anemia, thrombocytopenia; risk of monosomy 7 and myelodysplastic syndrome
SERKAL syndrome	*WNT4*	AR	46,XX DSD, renal dysgenesis, pulmonary hypoplasia
ACTH-resistance and related conditions			
FGD1	*MC2R* (ACTH receptor)	AR	Tall stature (pre-treatment)
FGD2	*MRAP* (MC2R-accessory protein)	AR	
Nicotinamide nucleotide transhydrogenase	*NNT*	AR	Early puberty
Thioredoxin reductase 2	*TXNRD2*	AR	Heart defects
Triple A syndrome (Allgrove syndrome)	*AAAS* (Aladin)	AR	Achalasia, alacrima, ataxia/neurological involvement, hyperkeratosis
Minichromosome maintenance-4	*MCM4*	AR	Natural killer cell defects, microcephaly, post-natal growth failure
Metabolic conditions			
Sphingosine-1-phosphate lyase 1 insufficiency	*SGPL1*	AR	Steroid-resistant nephrotic syndrome, ichthyosis, neurological involvement, hypothyroidism, cryptorchidism
X-linked adrenoleukodystrophy	*ABCD1*	X-linked	Neurological dysfunction
Zellweger spectrum disorders (incl. neonatal adrenoleukodystrophy)	*PEX* genes; related genes (Peroxins)	AR	Neurological, facial features, hepatic dysfunction
Mitochondrial disorders (Kearne-Sayre syndrome; Pearson syndrome; others)	Mitochondrial DNA, *MRPS7*, *NDUFAF5*, *GFER*	Maternal or AR	Variable multisystem features
Wolman disease	*LIPA* (Cholesterol ester)	AR	Failure to thrive, hepatosplenomegaly, adrenal calcification
Autoimmune conditions			
APS1 (APECED)	*AIRE* (Autoimmune regulator)	AD, AR	Hypoparathyroidism, mucocutaneous candidiasis, alopecia, pernicious anemia, other autoimmune features

CPHD, combined (multiple) pituitary hormone deficiency; AD, autosomal dominant; AR, autosomal recessive; CAH, congenital adrenal hyperplasia, DSD, differences (disorders) in sex development; SLD, sex-limited dominant; IUGR, intrauterine growth restriction; FGD, familial glucocorticoid deficiency. [a]Partial defects in STAR and P450scc can present with predominant glucocorticoid insufficiency in childhood and mimic FGD.

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loss usually results in hyperkalemia and hyponatremia at around 5–7 days of life, mandating urgent monitoring and treatment once the diagnosis is made. Boys (46,XY) with 21-OHD have no obvious signs at birth and usually present in a salt-losing adrenal crisis between 1 and 2 weeks of age, so many countries include 17-hydroxyprogesterone in their newborn screening program. More detailed reviews of CAH and its management are presented elsewhere (32, 33).

Adrenal Hypoplasia

Adrenal hypoplasia is an underdevelopment of the adrenal glands, which often presents with PAI in early life (**Figure 1B**). Usually this is an X-linked condition, or sometimes associated with intrauterine growth restriction (IUGR) (fetal growth restriction, FGR) syndromes.

X-Linked Adrenal Hypoplasia

X-linked congenital adrenal hypoplasia (adrenal hypoplasia congenita, AHC) primarily affects boys and is associated with disruption of the nuclear receptor, DAX-1 (encoded by *NR0B1*) (35, 36). This condition presents with salt-losing PAI in the first 2 months of life (40%), or more insidiously with AI in childhood (37). Late-onset forms of the condition have also been described (38–40).

X-linked AHC has three main features: PAI, hypogonadotropic hypogonadism (HH) in adolescence, and impaired spermatogenesis (41). Some boys may paradoxically have *macro*phallia at birth, and initial presentation with either isolated mineralocorticoid insufficiency or isolated glucocorticoid insufficiency is reported (42, 43). Growth hormone insufficiency has also been diagnosed in a small subset of boys (37, 44–46).

Boys with PAI and HH in adolescence almost invariably have X-linked AHC, especially if there is a family history of X-linked adrenal dysfunction. Even without such a history, we found that approximately 40% of boys presenting with salt-losing AI in the first two months of life had X-linked AHC, once more common conditions such as CAH had been excluded (47). Approximately two-thirds of boys have pathogenic missense or loss-of-function (stop gain, frameshift) variants in DAX-1/*NR0B1*, around one-sixth have a localized deletion of this gene on the X-chromosome (Xp21), and one-sixth have a larger Xp contiguous gene deletion syndrome that can involve genes causing glycerol kinase deficiency (*GKD*), ornithine transcarbamylase deficiency (*OTC*) and Duchenne Muscular Dystrophy (*DMD*) (47). Very rarely, girls have X-linked AHC due to skewed X-inactivation (48). Establishing this diagnosis early allows prompt recognition and management of both the PAI and potential associated conditions (39). Families can be counseled about risk in brothers or in the maternal family, and presymptomatic boys diagnosed (49).

Steroidogenic Factor-1 (SF-1/*N5A1*)

Steroidogenic factor-1 (SF-1/*NR5A1*) is a related nuclear receptor considered as a "master-regulator" of adrenal and reproductive development (36). Severe disruption of SF-1 has very rarely been associated with early-onset PAI in 46,XX girls and 46,XY phenotypic female babies with testicular dysgenesis, usually due to disruption of key DNA-binding elements of this transcription factor (50, 51). In contrast, more than 200 individuals and families with heterozygous pathogenic variants in SF-1/*NR5A1* have been reported, having a wide spectrum of reproductive phenotypes (from gonadal dysgenesis through to male factor infertility or primary ovarian insufficiency and ovotesticular DSD) (36, 52–54). To date, adrenal function is normal in most of these individuals.

IMAGe Syndrome (*CDKN1C* and *POLE1*)

AI associated with IUGR/FGR can occur as part of IMAGe syndrome (intrauterine growth restriction, metaphyseal dysplasia, adrenal hypoplasia, genitourinary anomalies) (55, 56). Children usually present with salt-losing PAI in early life. Other variable features include frontal bossing, impaired glucose tolerance, and hearing loss.

Classic IMAGe syndrome is associated with gain-of-function variants in the cell-cycle repressor, CDKN1C (56). This is a paternally-imprinted (maternally-expressed gene), so is usually inherited from the mother, but can occur *de novo*. IMAGe-associated pathogenic variants are localized within a very specific motif in the PCNA-binding motif of CDKN1C, causing impaired cell cycle S-phase progression (57). Variants neighboring this motif can cause IUGR/Russell-Silver Syndrome phenotypes with normal adrenal function (58, 59). Of note, loss-of-function of CDKN1C is associated with Beckwith-Wiedemann syndrome, an "overgrowth" syndrome, highlighting how different changes in one gene can have opposing phenotypes (58).

Recently, an "IMAGe-like" syndrome with AI and immunodeficiency (infections, lymphopenia, hypogammaglobulinemia) has been reported (60). These children have profound postnatal growth restriction, distinctive facial features, hip dysplasia and hypoplastic patellae. This condition results from pathogenic biallelic variants in polymerase epsilon-1 (*POLE1*, Pol ε), often involving a heterozygous intronic variant (c.1686 + 32C>G). POLE1 is a DNA polymerase that interacts with PCNA in S-phase DNA replication.

MIRAGE Syndrome (*SAMD9*)

Another multisystem growth restriction syndrome associated with adrenal hypoplasia is MIRAGE syndrome (myelodysplasia, infections, restriction of growth, adrenal hypoplasia, genital phenotypes, enteropathy) (61, 62). Infants with severe forms of MIRAGE are born preterm and develop salt-losing PAI in early life. Recurrent infections (viral, bacterial and fungal), anemia/thrombocytopenia, nephropathy, severe enteropathy, esophageal reflux and aspiration are common, and 46,XY children have penoscrotal hypospadias or gonadal (testicular) dysfunction with a female phenotype. Mortality is high and children who survive show long-term growth restriction. As many of these features are found in sick, preterm, growth-restricted babies, it is likely that this condition is under-diagnosed.

MIRAGE syndrome results from heterozygous gain-of-function missense mutations in the growth repressor, sterile alpha domain containing 9 (*SAMD9*) (61, 62). These changes usually occur *de novo* and restrict cell growth and division,

potentially through reduced recycling of growth factor receptors. SAMD9 also plays a role in innate viral immunity and host defense.

One interesting aspect of MIRAGE syndrome is how secondary genetic events can dynamically modify the phenotype through "revertant mosaicism." For example, development of progressive, somatic monosomy 7 in *cis* (i.e., on the same allele) "removes" the deleterious gain-of-function mutation in SAMD9 allowing a clonal growth advantage of these affected cells, especially in the hematopoietic system, and reversal of the postnatal anemia and thrombocytopenia (61, 62). However, monosomy 7 is linked to the development of myelodysplastic syndrome, which can lead to leukemia if other genetic changes occur. Interestingly, somatic *loss-of-function* (nonsense or frameshift) changes or uniparental disomy in *cis* can also "remove" the mutant allele and ameliorate the phenotype (5, 62–64). Increasingly, children with milder MIRAGE-like features are being reported, many with normal adrenal function (65).

SeRKAL Syndrome (*WNT4*) and Other Associations

SeRKAL syndrome (female sex reversal and dysgenesis of kidneys, adrenals, and lungs) has been reported in a single family with homozygous disruptive mutations in WNT4, a signaling molecule implicated in adrenal development (66). Other historic reports have rarely described AI with Pena–Shokeir syndrome type I (*DOK7*, *RAPSN*), pseudotrisomy 13, Galloway–Mowat syndrome (*WDR73*), Pallister–Hall syndrome (*GLI3*, with pituitary defects) and Meckel–Gruber syndrome (*MKS1*) (67).

ACTH Resistance-Like Conditions

Another important group of conditions causing PAI in childhood are ACTH-resistance conditions (also known as Familial Glucocorticoid Deficiency, FGD) and related disorders (68). Some of these may present in early infancy.

Familial Glucocorticoid Deficiency Type 1 (*MC2R*)

Familial Glucocorticoid Deficiency Type 1 (FGD1) is a recessive condition that results from pathogenic variants in the ACTH receptor (melanocortin 2 receptor, *MC2R*) (68, 69). Children sometimes present in the first weeks of life with signs of cortisol insufficiency (hypoglycemia/convulsions, prolonged jaundice) and marked hyperpigmentation. Genuine mineralocorticoid insufficiency is very rare, but transient salt loss or dilutional hyponatremia can occur, sometimes leading to a misdiagnosis of adrenal hypoplasia (70, 71). FGD1 can also present later in childhood with recurrent infections, hyperpigmentation, and lethargy. Generally, children respond very well to glucocorticoid replacement, but ACTH concentrations can be difficult to suppress.

Familial Glucocorticoid Deficiency Type 2 (*MRAP*)

A similar form of ACTH-resistance results from disruption of the melanocortin 2 receptor accessory protein, MRAP (72). MRAP traffics MC2R to the adrenal cell membrane surface, so disruption of its function (usually due to splicing defects in exon 3) impairs ACTH signaling (68, 73, 74). Affected children usually present with severe glucocorticoid insufficiency and hyperpigmentation in the first few months of life.

Disorders Associated With Oxidative Stress (*NNT*, *TNXRD2*)

Defects in nicotinamide nucleotide transhydrogenase (*NNT*) are a well-established cause of isolated PAI in children, and occasionally additional features such as early puberty have been reported (68, 75, 76). This condition mostly presents after 1 year of age but has been reported as early as 4 months of age. To date, defects in thioredoxin reductase 2 (*TNXRD2*) are reported in a single family (sometimes with cardiac defects), and present in mid- or later childhood (77).

Triple A Syndrome (Allgrove Syndrome)

Triple A syndrome is a well-established condition linking PAI ("Addison disease"), with alacrima and achalasia of the esophagus (78, 79). This condition results from disruption of the protein aladin (encoded by *AAAS*), a potential nucleoporin component that may also influence cellular stress (80–82). Alacrima is often present from birth but is difficult to diagnose. Other features usually develop in childhood, or in the second decade of life (83, 84). Progressive neurological and autonomic dysfunction can also co-occur, so this is an important diagnosis to consider.

Other Related Forms of PAI

Disruption of minichromosome maintenance 4 (*MCM4*) causes mild PAI together with short stature and immunodeficiency (85). To date, this is only reported in individuals of Irish Traveller ancestry, and typically manifests in mid-childhood. As noted above, partial (non-classic) high steroidogenic blocks (*STAR* and *CYP11A1*) can present in childhood with PAI. Non-classic STAR defects are sometimes termed FGD3 (27–31).

Metabolic Conditions

Several metabolic conditions are associated with PAI but the presentation and features can be variable (86).

Sphingosine-1-Phosphate Lyase (*SGPL1*) Deficiency

SGPL1 deficiency is a novel sphingolipidosis that results from impaired breakdown of sphingosine 1-phosphate (87–89). Key features are PAI (sometimes with adrenal calcifications) and steroid-resistant nephrotic syndrome (SRNS), as well as ichthyosis, neurological dysfunction, primary hypothyroidism, lymphopenia and undescended testes. Many children present with PAI in the first year of life (hyperpigmentation, or adrenal crisis), although some first present with SRNS and the use of steroid treatment may delay the adrenal phenotype. Other features are variable and can appear or progress with time.

Adrenoleukodystrophy and Related-Conditions

Adrenoleukodystrophy (ALD) is a very important cause of PAI because of associated progressive neurological features (90). The X-linked form of ALD due to defects in *ABCD1* usually presents in childhood, and sometimes with adrenal-only features. Thus, all boys with undiagnosed causes of PAI should have long-chain fatty acids measured and there are some calls for newborn screening to increase early detection, since allogenic hematopoietic stem cell transplantation may reduce the progression of cerebral X-ALD in patients with early stages of disease, and hematopoietic stem cell gene therapy has been investigated (91–94). In contrast, "neonatal adrenal leukodystrophy" is now

classified as part of the "Zellweger Spectrum Disorders" (with Zellweger syndrome/cerebrohepatorenal syndrome, infantile Refsum disease and rhizomelic chondrodysplasia punctata type 1) (95). This spectrum of disorders results from defects in peroxisomal function (13 different *PEX* genes and others) and has many features including hypotonia, seizures, hepatic dysfunction and renal cysts. PAI has been reported, usually in childhood or with an impaired stress response (96), so screening after 1 year of age has been recommended (95).

Mitochondrial Disorders

Mitochondrial defects have a range of causes and presenting features. Adrenal dysfunction occurs in rare cases, more often associated with large scale mitochondrial DNA deletions (e.g., Kearns-Sayre and Pearson syndromes), but also pathogenic variants in other related genes (e.g., *MK-TK*, *MRPS7*, *QRSL1*, *NDUFAF5*, *GFER*) (86, 97).

Wolman Disease

Wolman disease (primary xanthomatosis) results from disruption of lysosomal acid lipase (*LIPA*), and is associated with AI (often with adrenal calcifications), failure to thrive, hepatosplenomegaly and anemia in the first few months of life. It is a lysosomal storage disorder that is usually fatal, although improvements with enzyme replacement treatment (sebelipase alfa) are reported (98–100).

Autoimmune Conditions

Although autoimmune "Addison disease" is the most common cause of AI in adolescents and adults, autoimmune PAI is rare in children (101). The best-established condition is Autoimmune Polyglandular Syndrome type 1 (APS1, also known as APECED), due to defects in autoimmune regulator (*AIRE*). Early features can include mucocutaneous candidiasis and rarely hypoparathyroidism (hypocalcemia). PAI and other associations usually occur in childhood or later life.

Other Causes of PAI

Physical causes of AI such as hemorrhage or infiltration (e.g., neuroblastoma) should not be overlooked. Unilateral adrenal hemorrhages detected by imaging are common (1:200–500 newborn), but usually asymptomatic (102). Symptomatic bilateral hemorrhages are rare but can cause profound AI. As in older children, prolonged administration of glucocorticoids for other conditions can suppress the hypothalamo-pituitary adrenal (HPA) axis and cause AI if withdrawal is rapid.

Transient, relative AI has been described in some very preterm babies, or in sick newborn children under stress. The physiological basis of this is unclear, but steroid supplements have been used in some situations (103).

IMPORTANCE OF MAKING A SPECIFIC DIAGNOSIS

Making a specific genetic diagnosis has several benefits. It allows tailored treatment of the specific underlying hormonal defect (such as the need for ongoing mineralocorticoid replacement or not) and permits the surveillance, early recognition, and prompt treatment of associated extra-adrenal features (16, 61, 62, 70, 71, 87, 98).

Reaching a specific genetic diagnosis also has wider implications for the family, especially as these conditions have a range of inheritance patterns (e.g., autosomal recessive, dominant/*de novo*; X-linked, imprinted). This information guides genetic counseling during future pregnancies, and potentially allows pre-symptomatic diagnosis and treatment in relatives with subclinical disease (49).

GENETIC TESTING FOR PAI IN EARLY LIFE

Traditionally, genetic testing has relied on Sanger sequencing of *candidate genes* one at a time. This approach may still have a role in common conditions such as 21-OHD (*CYP21A2*), where there is a specific biochemical profile, well-established pathogenic variants, and a pseudogene that can complicate analysis, or in X-linked AHC (DAX-1/*NR0B1*) when well-established associations (e.g., HH) or inheritance patterns (e.g., X-linked) are present.

However, associated features or pathognomonic biochemical patterns are often not present when an infant presents with PAI, so "next generation" sequencing (NGS) approaches are increasingly time- and cost-effective.

Access to services varies from country to country, but *targeted "panels"* to analyze all key PAI genes at once are increasingly available as a clinical service. In addition, several studies have shown how "trio" *whole exome sequencing* (WES) can help diagnose sick infants and children, especially when there are complex, multisystem features, and exome analysis has been reported to help in the diagnosis of children with PAI (104). *Whole genome sequencing* (WGS) will become increasingly available and has potential advantages and disadvantages at present compared to panels/WES.

In general, genetic testing for PAI has a high diagnosis rate, certainly when compared to other pediatric endocrine conditions such as congenital hypothyroidism and hypothalamo-pituitary hormone deficiencies. For example, in a national cohort study of rare, undiagnosed PAI in Turkey (with CAH and obvious metabolic causes excluded), a specific genetic diagnosis was reached in 80–90% of children (105), although the diagnostic yield of autosomal recessive conditions was high due to high consanguinity rates. New genetic causes may still emerge as our understanding of human adrenal development expands (106).

Finally, founder effects and genetic "hotspots" can be very important in identifying a specific genetic cause of PAI and taking a history of family ancestry is key. For example, in Turkey the *MRAP* splice variant cIVS3ds + 1delG is found in the West, whereas P450scc/CYP11A1 p.R451W occurs in Eastern regions (105). As families migrate around the world, founder effects are seen in children born in other countries. Knowing a specific hotspot can allow focused and cost-effective screening of "at risk" family members before the onset of PAI.

CONCLUSIONS

AI is an important diagnosis to consider in any sick newborn infant and prompt investigation and treatment is essential. Genetic testing is increasingly useful for finding a specific cause, predicting associated features, counseling families and, in some situations, for modifying treatments.

AUTHOR CONTRIBUTIONS

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REVIEW
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Congenital Adrenal Hyperplasias Presenting in the Newborn and Young Infant

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Congenital adrenal hyperplasia includes autosomal recessive conditions that affect the adrenal cortex steroidogenic enzymes (cholesterol side-chain cleavage enzyme; 3β-hydroxysteroid dehydrogenase; 17α-hydroxylase/17,20 lyase; P450 oxidoreductase; 21-hydroxylase; and 11β-hydroxylase) and proteins (steroidogenic acute regulatory protein). These are located within the three major pathways of the steroidogenic apparatus involved in the production of mineralocorticoids, glucocorticoids, and androgens. Many countries have introduced newborn screening program (NSP) based on 17-OH-progesterone (17-OHP) immunoassays on dried blood spots, which enable faster diagnosis and treatment of the most severe forms of 21-hydroxylase deficiency (21-OHD). However, in several others, the use of this diagnostic tool has not yet been implemented and clinical diagnosis remains challenging, especially for males. Furthermore, less severe classic forms of 21-OHD and other rarer types of CAHs are not identified by NSP. The aim of this mini review is to highlight both the main clinical characteristics and therapeutic options of these conditions, which may be useful for a differential diagnosis in the neonatal period, while contributing to the biochemical evolution taking place in the steroidogenic field. Currently, chromatographic techniques coupled with tandem mass spectrometry are gaining attention due to an increase in the reliability of the test results of NPS for detecting 21-OHD. Furthermore, the possibility of identifying CAH patients that are not affected by 21-OHD but presenting elevated levels of 17-OHP by NSP and the opportunity to include the recently investigated 11-oxygenated androgens in the steroid profiles are promising tools for a more precise diagnosis and monitoring of some of these conditions.

Keywords: newborn, 21-hydroxylase deficiency, 11-hydroxylase deficiency, 20–22-desmolase deficiency, StAR deficiency, P-450 oxydoreductase deficiency, 3-beta hydroxysteroid dehydrogenase deficiency, 17-hydroxylase/17-20 lyase deficiency

INTRODUCTION

The most common and representative example of the congenital adrenal hyperplasia (CAH) group of disorders (≥90%) is the 21-hydroxylase deficiency (**CYP21A2-D**). Less frequent types of CAH are 11β-hydroxylase deficiency (**CYP11B1-D**, up to 8% cases), 17α-hydroxylase/17–20 lyase deficiency (**CYP17A1-D**), 3β-hydroxysteroid dehydrogenase deficiency (**HDS3B2-D**), P450 oxidoreductase deficiency (POR-D), P450 cytochrome side-chain cleavage deficiency (**CYP11A1-D**), and StAR deficiency (**StAR-D**). In CYP21A2-D and CYP11B1-D, only adrenal steroidogenesis is affected, whereas a defect in the other enzymes also involves gonadal steroid biosynthesis (1, 2) (**Table 1**).

Steroid Acute Regulatory Protein deficiency—Lipoid CAH (StAR-D)

Epidemiology/Genetics

StAR-D is uncommon in most populations, but it is relatively more frequent in East Asian (3, 4), Arab (5), and Swiss (6) populations because of the occurrence of the p.Q258X, p.R182L/p.R182I, and p.L260P founder mutations, respectively. To date, ∼85 pathogenic variants of the *StAR* gene have been reported (www.hgmd.cf.ac.uk) (**Table 1**).

Essential Biochemistry

StAR is a fundamental actor in steroidogenesis, transferring cholesterol from the outer (OMM) to the inner mitochondrial membrane (IMM), where CYP11A1 can convert cholesterol to pregnenolone (Preg) (**Figure 1A**). The complex pathophysiology of StAR-D is explained by the "two-hit disease model" (5): the major part of steroidogenesis is StAR dependent, and its deficit, the first hit, activates the ACTH axis and *de novo* cholesterol biosynthesis; the consequent steroid underproduction due to the toxic effects of accumulating cholesterol follows as the second hit. The impairment of testicular steroidogenesis, which is active earlier than the ovarian one, is the first consequence of StAR-D with fetal androgen deficiency, causing undervirilization in 46,XY genetic newborns (7). Fetal adrenal androgen deficiency also leads to reduction of maternal estriol (E3) levels, prenatally measurable in a maternal urine sample (8). As placental steroidogenesis is not StAR dependent, Prog production is able to maintain pregnancy to term.

Clinical Presentation and Diagnosis

In its most severe form, the affected newborns cannot produce significant amounts of any steroid (9, 10). They show high ACTH levels, increased plasma renin activity, and engorged adrenal glands containing excessive amounts of cholesterol and its derivatives (5) (**Table 1**). Classic patients have severe salt loss within the 1st months of life and female external genitalia, irrespective of chromosomal sex (11). In 46,XY babies, Sertoli cells stay intact and the anti-Mullerian hormone (AMH) inhibits the development of Mullerian structures. The hydrosaline balance is controlled prenatally by the placenta, but mineralocorticoid (MC) deficiency emerges within 2–3 weeks due to progressive cellular destruction and some remaining StAR-independent MC biosynthesis. "As the ovary is steroidogenically quiescent until puberty, it is protected from cellular damage until steroidogenesis begins" (12, 13).

P450 Cytochrome Side-Chain Cleavage Deficiency (CYP11A1-D)

Epidemiology/Genetics

CYP11A1-D is an even rarer defect than StAR-D, and it is caused by pathogenic variants of the *CYP11A1* gene (14). To date, 40 patients and 25 variations of *CYP11A1* have been reported. Almost all cases are homozygous or compound heterozygous (15). Autosomal dominant inheritance has also been proposed in a few cases (16, 17).

Essential Biochemistry/Pathophysiology

CYP11A1 catalyzes the conversion of cholesterol to Preg in three consecutive rate-limiting steps: 20α-hydroxylation, 22R-hydroxylation, and cleavage of the C20–C22 carbon side chain (18) (**Figure 1A**). CYP11A1-D determines defects in all three steroidogenic pathways: MC, glucocorticoid (GC) in the adrenals, and androgen (Andr) in the adrenals and gonads. Complete CYP11A1-D was considered incompatible with term pregnancies due to impaired placental progesterone and maternal estrone (E1) production; the reason why some cases survived pregnancy is still not completely clear (19–22). The expression of CYP11A1-D occurs early in fetal testes, causing defective gonadal steroidogenesis that dramatically impairs virilization of 46,XY fetuses (23).

Clinical Presentation and Diagnosis

In newborns, the most severe presentation is characterized by early adrenal insufficiency with salt wasting (SW), hypoglycemia, skin hyperpigmentation, and complete feminization of external genitalia, regardless of sex chromosomes. The 46,XY newborns show normal or hypoplastic derivatives of Wolffian duct and small testes, whereas derivatives of Mullerian duct are absent (11). Histology of the testes reveals immature tissue without germ cells (20). A phenotype with neonatal and transient adrenal insufficiency, life-threatening failure to thrive, and normal male external genitalia in 46,XY patients was reported in 2018

Abbreviations: Δ4A, Δ4-androstenedione; 11KΔ4A, 11-keto-androstenedione; 11KT, 11-keto-testosterone; 17OHP, 17α-hydroxyprogesterone; 17OHPreg, 17α-hydroxypregnenolone; Aldo, aldosterone; AMH, anti-Mullerian hormone; Andr, androgens; B, corticosterone; CAH, congenital adrenal hyperplasia; CYP11A1-D, P450 cytochrome side-chain cleavage deficiency; CYP11B1-D, 11β-hydroxylase deficiency; CYP17A1-D, 17α-hydroxylase/17-20 lyase deficiency; CYP21A2-D, 21-hydroxylase deficiency; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; DOC, deoxycorticosterone; E1, estrone; E2, estradiol; E3, estriol; F, cortisol; EGS, External Genitalia Score; EMS, External Masculinization Score; GC, glucocorticoid; HDS3B2-D, 3β-hydroxysteroid dehydrogenase deficiency; IMM, inner mitochondrial membrane; LC/MSMS, liquid chromatography/tandem mass spectrometry; MC, mineralocorticoid; NIPD, non-invasive prenatal diagnosis; NSP, newborn screening program; OMM, outer mitochondrial membrane; POR-D, P450 oxidoreductase deficiency; Preg, pregnenolone; Prog, progesterone; S, 11-deoxycortisol; StAR-D, steroidogenic acute regulatory deficiency; SV, simple virilising; SW, salt wasting; T, testosterone; THDOC, tetrahydro-deoxycorticosterone; THS, tetrahydro-deoxycortisol; US, ultrasound.

TABLE 1 | A summary of genetic, early clinical, biochemical features, and therapy of the CAH deficiencies presenting in the 1st year of life [modified by (1)].

			STAR PROT OMIM 201710	CYP11A1 OMIM 118485	HSD3B2 OMIM 201810	CYP17A1 OMIM 202110	CYP21A2 OMIM 201910	CYP11B1 OMIM 202010	POR OMIM 201750
Genetics		Gene	***StAR***	***CYP11A1***	***HSD3B2***	***CYP17A1***	***CYP21A2***	***CYP11B1***	***POR***
		Locus	Chr. 8p11.23; 7 exons	Chr. 15q24.1; 9 exons	Chr. 1p12; 4 exons	*Chr.* 10q24.32; 8 exons	Chr. 6p21.33; 10 exons	Chr. 8q24.3; 9 exons	Chr. 7q11.23; 17 exons
Clinical/ biochemical features at birth	MC	Renin	↑↑	↑↑	↑↑	↓	↑↑	↑↓	↓↑
		Na/K	↓/↑	↓/↑	↓/↑	↔	↓/↑	↔,↓/↔, ↑	↔
		BP	↓	↓	↓	↑ (no in partial defects)	↓	↑	↑
		Neonatal SW	+++	+++	+++	–	+++	–	–
	GC	Neonatal AI	+++	+++	+++	±	+++	+++	++
		Hypoglycemia	++	++	++	–	±	–	–
	Andr	Genitalia	46,XY DSD	46,XY DSD	46,XY DSD; 46,XX DSD (mild in 25% of cases)	46,XY DSD; absence of secondary sexual characteristics in both sexes	46,XX DSD	46,XX DSD	46, XY DSD; 46,XX DSD 75% of cases
Other features	Adrenal	Gland size	↑↑	↓↓	↔	↔	↑↑	↑ ↔	↔
Biochemical diagnostic markers		MC	↓↓	↓↓	Normal/↓	Serum: ↑ DOC Urine:↑ MC/ GC and ↑ androgens/ GC metabolites	↓/↔/↑*	Serum: ↑ S and DOC Urine: ↑ THS, THDOC	Normal
		GC	↓↓	↓↓	↓	Serum ↑ B	Serum: ↑ 21-DOF; urine:21-DOF (P'TONE)	Serum ↓ F	Normal
		Andr	↓↓	↓↓	Serum: ↑ stimulated ratio of Δ4 over Δ5 steroids; Urine: ↑ ratios DHEA/GC Metabolites and 5PT/GC Metabolite*	↓↓	Serum: ↑ 17-OHP ↑ 11-KΔ4A, 11-KT Urine: ↑17HP,PT Saliva: ↑11-KΔ4A and 11-KT	↑	Blood: mild ↑ 17-OHP; ↑ Pregn, Prog, 17-OHP Urine: combined impairment of diagnostic ratios for CYP17A1 and CYP21A2; ↑ of Pregn metabolites (PD)
Therapy		Hydrocortisone	+	+	+	+	+	+	±
		Fludrocortisone	+	+	+	–	+	±	–
		Mineralocorticoid receptor antagonists	–	–	–	±	–	+	–

*MC, mineralocorticoids; GC, glucocorticoids; Andr, androgens; K, potassium; BP, blood pressure; SW, salt wasting; AI, adrenal insufficiency. *Cross reaction with high levels of other adrenal steroids. For steroid abbreviations, see the specific table. *It can be notoriously difficult to diagnose HSD3B2-D on urine sample alone due to the naturally high levels of 3βOH5ene steroids in neonates.*

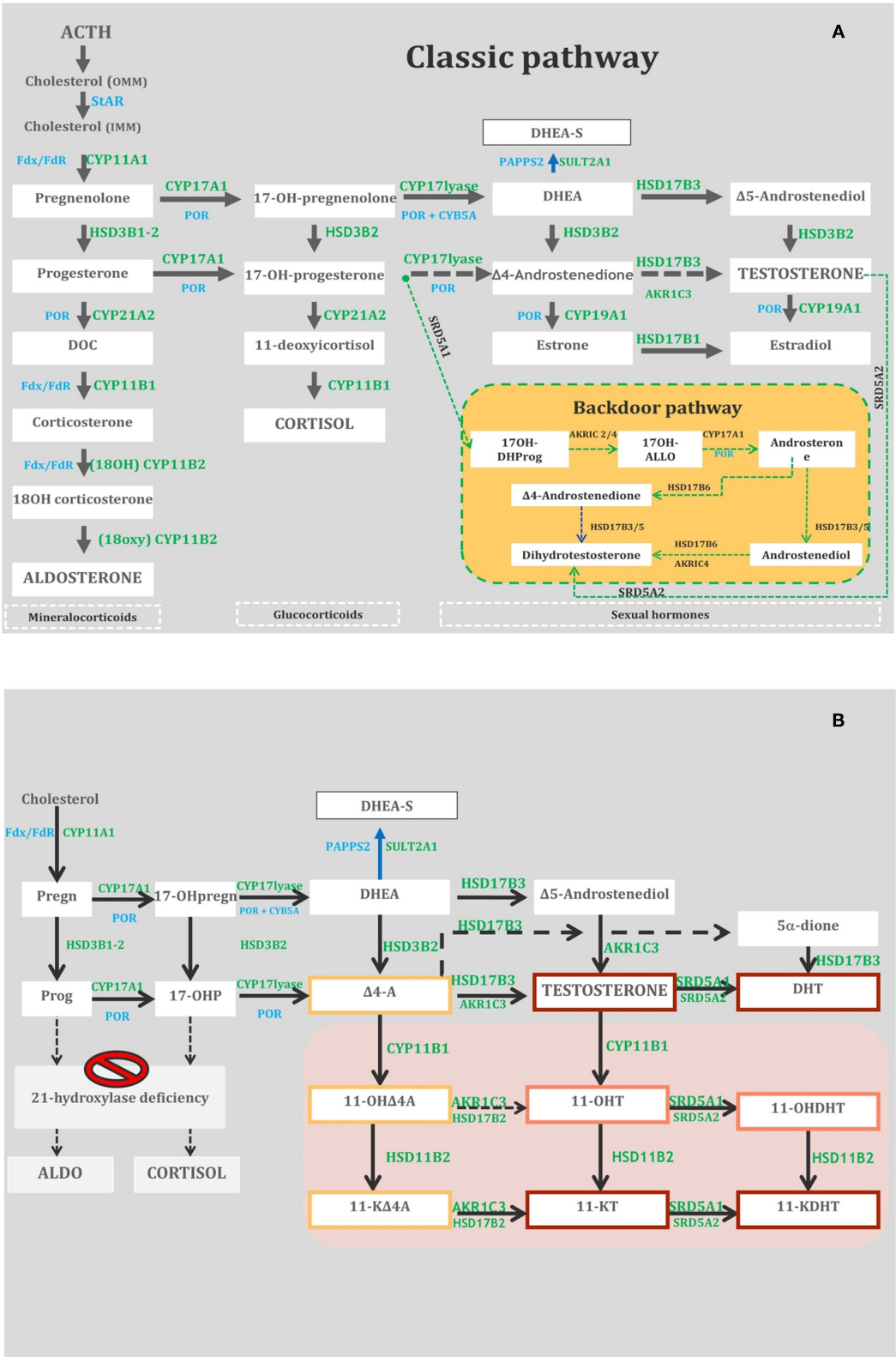

FIGURE 1 | (A) Adrenal and gonadal steroidogenesis (Classic and Backdoor pathways). StAR, steroidogenic acute regulatory protein; OMM, outer microsomal membrane; IMM, inner microsomal membrane; DHEA/DHEA-S, Dehydroepiandrosterone/D-Sulfate; DOC, Deoxycorticosterone; 17OH-DHProg, 5-Pregnan-17-ol-3,20-dione (diol); 17OH-ALLO, 17OH-allopregnanolone; CYP11A1, cholesterol side-chain cleavage enzyme; CYP17A1, 17-hydroxylase/17, 20

(Continued)

FIGURE 1 | lyase; SULT2A1, dehydroepiandrosterone (DHEA) sulfotransferase; POR, P450 oxidoreductase; CYP21A2, 21-hydroxylase; HSD3B2, 3-hydroxysteroid dehydrogenase; CYP11B1, 11-hydroxylase; CYP11B2, aldosterone synthase; HSD17B3, 17-hydroxysteroid dehydrogenase type 3; SRD5A1, 5-reductase type 1; SRD5A2, 5-reductase type 2; Fdx/ FdR, ferredoxin/ferredoxin reductase; CYB5A, cytochrome b5. **(B)** The metabolic pathways of classic and non-classic androgens. The gray box indicates 11-oxygenated C19 steroids. The red, orange, and yellow boxes depict steroids with strong, mild, and weak androgenic activities, respectively. HSD17B3, 17-hydroxysteroid dehydrogenase type 3; HSD3B2, 3-hydroxysteroid dehydrogenase type 2; AKR1C3, aldo-keto reductase family 1 member C3; CYP11B1, cytochrome P450 11B1; HSD11B1, 11-hydroxysteroid dehydrogenase type 1; HSD11B2, 11-hydroxysteroid dehydrogenase type 2; HSD17B2, 17-hydroxysteroid dehydrogenase type 2; SRD5A1, 5-reductase type 1; SRD5A2, 5-reductase type 2; Pregn, pregnenolone; 17-OHpregn, 17-OHpregnenolone; DHEA-S, dehydroepiandrosterone-sulfate; SULT2A1, sulfotransferase family 2A member 1; DHEA, dehydroepiandrosterone; Prog, progesterone; 17-OHP, 17-OH-progesterone; D4-A, androstenedione; 11-OHD4A, 11-hydroxyandrostenedione; 11-KD4A, 11-ketoandrostenedione; 11-OHT, 11-hydroxytestosterone; 11-KT, 11-ketotestosterone; 5-dione, 5-androstanedione; DHT, dihydrotestosterone; 11-OHDHT, 11-hydroxydihydrotestosterone; 11-KDHT, 11-ketodihydrotestosterone.

in three heterozygous related cases (17). One case of mid-shaft hypospadias and cryptorchidism at birth and another with penoscrotal hypospadias associated with late-onset adrenal insufficiency (9 and 2 years of age, respectively) were reported in 2009 (24) and 2012 (25). In newborns, blood tests showed severe hyponatremia, hyperkalemia, extremely elevated levels of ACTH, and renin activity with low or inappropriately normal levels of cortisol and aldosterone. Unlike most classic lipoid-CAH (26), adrenal glands are reduced in size in CYP11A1-D (27).

3β-Hydroxysteroid Dehydrogenase Deficiency (HSD3B2-D)

Epidemiology/Genetics

HSD3B2-D is a very rare form of CAH (estimated incidence of < 1/1,000,000 live births) (18, 28) caused by mutations in the *HSD3B2* gene (**Table 1**) that encode the 3β-HSD2 enzyme. It is involved in all three steroidogenic pathways: aldosterone, cortisol, and androgen precursors in the adrenals and testosterone (T) in the gonads (18). Loss-of-function mutations (<5% residual enzyme activity) predict the neonatal SW phenotype. Mutations causing >5% 3β-HSD2 activity lead to residual MC production without SW (29).

Essential Biochemistry/Pathophysiology

3β-HSD2 enzyme converts Δ5-3β-hydroxysteroids into corresponding Δ4-3-keto isomers, Preg to Prog, 17α-hydroxypregnenolone (17OHPreg) to 17α-hydroxyprogesterone (17OHP), dehydroepiandrosterone (DHEA) to Δ4-androstenedione (Δ4A), and androstenediol to T. In SW HSD3B2-D, glucocorticoid and mineralocorticoid are impaired causing hyponatremia, hyperkalemia, and elevated renin concentrations in both sexes. In females, 3β-HSD2 deficiency prevents the flooding of 17OHP and Δ4A to backdoor and 11-oxyandrogen production pathways (see CYP21A2-D) (**Figures 1A,B**); in males, T production is impaired during the critical period of sexual differentiation and dihydrotestosterone (DHT) production is subsequently reduced by classical and backdoor pathways (30).

Clinical Presentation and Diagnosis

Historically, the clinical presentation of HSD3B2-D at birth is described as the "classic form," with or without SW, hypoglycemia, ambiguous genitalia, and hypogonadism in both sexes. Recent studies have shown that HSD3B2-D rarely causes ambiguous genitalia in females and thus the affected 46,XX newborns may present mild clitoromegaly only, whereas affected 46,XY newborns may present some degree of external genitalia undervirilization or isolated hypospadias, which need to be graded based on reliable tools (EGS) (31), (EMS) (32), (Prader) (33). The frequency of HSD3B2-D could be underestimated in females without SW and normal genitalia. However, in countries with NSP for 21-OHD, it is possible that newborns with HSD3B2-D may show false positivity for elevated levels of 17-OHP (34–37). The principal diagnostic test for HSD3B2-D is the serum measurement of 17-OHpreg, cortisol, Δ4A, 17-OHP, and DHEA (basal or post-ACTH stimulation) (28) with a predominance of Δ5 steroids (i.e., Preg, 17OHPreg, and DHEA) over Δ4 steroids (Prog, 17OHP, and Δ4A). Guran et al. (30) reported that high baseline 17OHpreg-to-cortisol ratio and low 11-oxyandrogen concentrations by LC/MSMS provide an unequivocal biochemical diagnosis of patients with HSD3B2-D. Although urinary steroid profiling is considered to be similarly accurate and less invasive for diagnosis (38), it can be notoriously difficult to diagnose HSD3B2-D on urine sample alone due to the naturally high levels of 3βOH5ene steroids in neonates.

17α-Hydroxylase/17,20 Lyase Deficiency (CYP17A1-D)

Epidemiology/Genetics

The incidence of CYP17A1-D is estimated to be about 1 in 50,000 (39). The disease prevalence is higher in certain countries such as the Netherlands (Friedlanders), Brazil, China, and Japan, where it is the second leading cause of CAH. This is attributable to loss-of-function mutations in the *CYP17A1* gene (40) (**Table 1**). Over 100 mutations in the *CYP17A1* gene are known, mostly resulting in complete loss of enzymatic activities of both 17-hydroxylase and 17–20 lyase (39). Researchers have also reported partial loss of enzymatic activity and loss of either hydroxylase or lyase activity alone (41).

Essential Biochemistry

CYP17A1 mediates three major transformations in cortisol and sex steroid biosynthesis. In particular, 17-hydroxylase mediates the synthesis of 17-Preg from Preg and 17OHP from Prog, whereas 17–20 lyase controls the production of DHEA from 17OHPreg. This latter step is of paramount importance as DHEA is the progenitor of steroid sex hormones (**Figure 1A**). The biochemical markers used to diagnose CYP17A1-D are shown in **Table 1**.

Clinical Presentation and Diagnosis

In 46,XX patients, external genitalia are normal on the neonatal exam as are the internal one to ultrasound (US). They may have no complaints before the typical age of puberty when the deficiency in sex hormones becomes apparent and they may develop hypertension or hypokalemia and high gonadotropin levels (hypergonadotropic hypogonadism).

In 46,XY patients, the presentation is typically under masculinization and can range from phenotypic female to ambiguous or small male genitalia (41). On physical examination, they may have a blind pouch instead of a vagina with a lack of internal female genitalia. The testes are undescended or located in the inguinal canal on imaging studies. Early diagnosis and treatment allow for the prevention of morbidity associated with hypertension, electrolyte abnormalities, and impairment of sexual development. As NSP identifies classic CYP21A2-D but does not detect CYP17A1-D, provider awareness and consideration of this condition are imperative for appropriate diagnosis.

21-Hydroxylase Deficiency (CYP21A2-D)

Epidemiology/Genetics

The most common form of CAH is represented by CYP21A2-D (90% cases). The severity of the enzymatic deficiency determines three clinical forms: SW (<1% enzyme activity), simple virilizing (SV; 1–2%), and non-classical (NC; 20–60%). The incidence of classic forms (SW and SV) ranges between 1 in 13,000 and 1 in 15,000 live births (42): in most populations, the frequency of heterozygous carriers is 1 in 60. CYP21A2-D is caused by mutations in the *CYP21A2* gene (6p21.3). Microconversions or apparent gene conversions that cause the transfer of an inactive pseudogene (CYP21P) to the functional gene are responsible for 95% of pathogenic variations (43). Rare patients with classic CAH (SW) show a "contiguous gene syndrome", with CAH and Ehlers–Danlos Syndrome (EDS) features, which is called "CAH-X" (44).

Essential Biochemistry/Pathophysiology

In SW CYP21A2-D, GC, and MC production is severely impaired, whereas abnormal amounts of Andr are produced, stimulated by the increased levels of ACTH. 17OHP elevation represents the hallmark of the disease, and the large majority of classic CYP21A2-D patients show basal levels of 17OHP as >300 nmol/L (>10,000 ng/dL) (45). 17OHP is converted to T and 5α-DHT, two androgens with potent activity, by the so-called "front door" pathway and directly to DHT *via* an alternative pathway known as "the backdoor pathway" (46, 47) (**Figure 1A**). The latter could lead to hyperandrogenism less responsive to GC treatment (48). 11-Keto-testosterone (11KT) is derived from 11-hydroxylation of Δ4A and T by CYP11B1 and acts as a potent androgen with a fundamental role in the pathophysiology of classic CYP21A2-D (49) (**Figure 1B**). It could be utilized in the future as a more precise biochemical marker of the disease (measured by means of LC/MSMS) than DHEA, Δ4A, and T (49, 50).

Neonatal Presentation

All fetuses affected by classic CAH show varying degrees of genital virilization due to exposure to intrauterine androgen excess, so that any newborn with ambiguous genitalia or in extreme cases apparently male genitalia and non-palpable gonads (45) should be suspected of having SW CAH (2, 45). Patients with 46,XX very often show a vagina that opens into a common urogenital sinus with enlarged clitoris and normal cervix, uterus, and ovaries; 46,XY children may show macrogenitosomia and genital hyperpigmentation but are generally unrecognized at birth. Sodium loss and potassium retention occur in newborns with SW CAH, due to mineralocorticoid deficiency. This may be detected biochemically from 4 to 7 days of life, but takes longer to present clinically (2nd week to 1st month of life).

Newborn Screening

In several countries, NBS has been developed for early diagnosis of CYP21A2-D by measuring 17OHP blood levels on dried blood spots. NBS is fundamental in preventing SW crises in males and male sex assignment in affected females. The diagnosis of CYP21A2-D is made when 17OHP levels are above the cutoff levels that should be elaborated and adjusted for gestational age at each screening center (51). A second-tier test on the same blood sample by LC/MSMS multi-hormonal profile could improve the positive predictive value of the CAH screening (52) and be helpful in diagnosing other rarer forms of CAH (35, 53).

Prenatal Treatment

The prenatal diagnosis of affected CAH fetuses is usually made by chorionic villous sampling at 10–12 weeks of gestation or by amniocentesis at 15–16 weeks of gestation. Treatment in utero of potentially affected CAH patients is feasible by administering dexamethasone to the mother starting from the first weeks of pregnancy, with the aim of containing adrenal hyperandrogenism by reducing ACTH hypersecretion and avoiding genital masculinization in the CYP21A2-D female fetuses (2). However, it should still be considered an experimental therapy due to potential adverse effects on both unaffected children that need to be treated until diagnosis is achieved and their mothers (45). A non-invasive method using cell-free fetal DNA in maternal plasma (NIPD at 5 weeks of gestation) could allow selective treatment in affected females only (54) but is not routinely performed due to its complexity and associated cost.

11β-Hydroxylase Deficiency (CYP11B1-D)

Epidemiology/Genetics

11-OHD is among the most common causes of CAHs in the world, after 21-OHD, and accounts for about 5% of CAH patients with a European ancestry (55) and for about 15% of CAH patients in the Muslim and Jewish Middle Eastern populations (56). The classical form of 11-OHD has an estimated frequency of 1 in 200,000 live births (57). This is caused by mutations in the *CYP11B1* gene (**Table 1**). Approximately 130 mutations of the *CYP11B1* gene have been described so far.

Essential Biochemistry

In the normal adrenals, 11β-hydroxylase is expressed in the zona fasciculata and converts 11-deoxycortisol to cortisol in response to ACTH. 11β-hydroxylase and aldosterone synthase can convert DOC into corticosterone (B). 11-OHD disrupts the synthesis of cortisol with normal production of aldosterone (1). The key steroid used in diagnosis for the classic form is elevated 11-deoxycortisol basal levels (27). Serum B, DOC, and 17-OHP are also elevated, and elevated levels of the latter can cause CYP21A2-D misdiagnosis. The urinary metabolites, such as tetrahydro-cortisone, tetrahydro-11-deoxycorticosterone, and tetrahydro-11-deoxycortisol (2), are useful for diagnosis.

Clinical Presentation and Diagnosis

The classical form is characterized by excess androgen and hypertension. ACTH excess due to cortisol deficit causes overproduction of androgens and DOC: androgens lead to virilization similar to CYP21A2-D in affected female patients (46,XX DSD); excess of DOC causes low-renin hypertension (2). Hypertension might not be apparent during the neonatal period (in about one-third of patients) due to mineralocorticoid resistance, and some patients can present with salt loss during the neonatal period, especially after the start of the GC treatment (58).

P450 Oxidoreductase Deficiency (POR-D)

Epidemiology/Genetics

POR-D was first described in 2004 (59) as a rare form of CAH. Currently, about 100 cases of POR-D have been reported worldwide with a broad clinical spectrum, and most occurring in neonates and children (60). Since 2004, some pathogenetic variants causing defective binding of co-factors and others causing altered interaction with partner proteins have been described (61). The homozygous null mutations appear to be lethal (62, 63).

Essential Biochemistry

POR is involved in the metabolism of drugs and steroid hormones because "all cytochrome P450 enzymes located in the endoplasmic reticulum get electrons for their catalytic activities from the co-factor" (61) NADPH through POR (64, 65). The main microsomal POR-dependent enzymes are involved in the biosynthesis of steroid hormones in both the adrenal cortex and gonads (CYP17A1, CYP19A1, CYP21A2, and CYP15A1), as well as in the metabolism of drugs and endogenous substrates (CYP3A4 and CYP2D6) in the liver (66–68). Several studies also suggest a possible role for POR in bone development and retinoic acid metabolism, which lead to skeletal anomalies (60).

Clinical Presentation and Diagnosis

POR-D was initially identified as a difference of sex development (DSD) with ambiguous genitalia similar to some cases of Antley–Bixler syndrome (ABS), a bone malformation syndrome due to the presence of mutations in FGFR2 (69). A recent review (60) meta-analyzed the phenotypic features in newborns with POR-D, with DSD at birth in 69% of patients (78% 46,XX and 60% 46,XY) (60). Maternal virilization during pregnancy, due to a defect in aromatase (CYP19A1) activity, is described in 21% of mothers, with the highest incidence (44.4%) when at least one of the mutations was R457H (60).

Skeletal malformations resembling ABS features were described in the 84% of PORD patients without mutations in *FGFR2* (60), such as midface hypoplasia (71%), large joint synostosis (69%), craniosynostosis (65%), hand and feet malformations (61%), and bowing of the femora (21%) (63). A latent form of adrenal insufficiency rarely becomes clinically evident in the neonatal period (70–72). "Due to a very complex effect on steroid metabolism, it is preferable to diagnose PORD by performing mass spectrometry analysis of urine and blood samples" (61). Hormonal analysis is characteristic of mild to moderate increase in 17OH progesterone levels (found through neonatal screening or biochemical analysis), normal baseline ACTH, and cortisol levels with an inadequate increase in cortisol production after ACTH stimulation, normal values for renin and aldosterone, and elevated values for progesterone, corticosterone, 18OH corticosterone, 11-deoxycorticosterone (DOC), 18OH DOC, and 21-deoxycortisol (59). Low E3 and increased metabolites of Preg in urine or amniotic fluid of the mother can be useful for prenatal diagnosis (73). However, definitive diagnosis of PORD needs to be done by genetic analysis of the *POR* gene.

THERAPEUTIC APPROACH

Glucocorticoids (GCs)

Substitutive treatment with oral hydrocortisone (10–15 mg/m^2/day, divided into three daily doses) is mandatory for all classic forms of CAH presenting during the neonatal period. In CYP21A2-D and CYP11B1-D, GC administration prevents further genital virilization. Higher doses (15–30 mg/m^2/day, divided into 3 or 4 daily doses) are often indicated both initially, to slow down the excessive production of potentially unfavorable metabolites (21OHD), and subsequently, as neonates and young infants often require higher doses per surface area than older children or adults. Other forms of GC are not recommended due to possible ineffectiveness (cortisone acetate) or detrimental effects on child growth (prednisolone and dexamethasone). Alawi et al. (28) suggested administering hydrocortisone at slightly higher doses (12–18 mg/m^2/day) in HSD2B3-D due to the greater difficulty in suppressing androgens.

We recommend educating parents and caregivers for adrenal crisis prevention and at least doubling the dose of GC (but not MC) for situations such as febrile illness (>38.5°C) and gastroenteritis with dehydration. Parenteral hydrocortisone administration (i.v. bolus of 12.5 mg in neonates and young infants, often with glucose and saline and administered within 3–10 min; bolus repetition every 4–6 h in the first 24 h or a continuous infusion of 100 mg/m^2/day) is mandatory in cases of adrenal crisis with vomiting, major surgery accompanied by general anesthesia, and major trauma.

In patients <18 months of age, close monitoring in the first 3 months of life and every 3 months thereafter is recommended (45).

Mineralocorticoids (MCs)

Fludrocortisone (0.05–0.2 mg, once or twice daily) together with sodium chloride administration during the first 6–12 months of life (5 mmol/kg/day, divided into 4–5 meals; 17 mmol or mEq = 1 g NaCl) aims to prevent adrenal crisis in StAR-D, CYP11A1-D, SW HSD2B3-D, and SW CYP21A2-D. Monitoring of K and BP levels is important for dose management.

Other Medical Treatments

In undervirilized 46,XY newborns with StAR-D, CYP11A1-D, CYP17A1-D, or POR-D reared as male, sex steroid replacement (T or DHT) might be useful during minipuberty (74). In newborns with CYP11B1-D or CYP17A1-D, if blood pressure control is not achievable by glucocorticoids alone, then appropriate antihypertensives should also be administered. Sometimes, treatment with mineralocorticoid receptor antagonists may be necessary (75). In POR-D, the supplementation of sex steroids and glucocorticoids must be based on the steroid profile of the patient, considering the possibility of impaired drug metabolism. Skeletal malformations require orthopedic management. Potential therapeutic options include the introduction of external flavin (66) and treatment with cysteamine in case of arginine to cysteamine mutations (76).

Surgical Treatment

A multidisciplinary team with competence in DSD management is recommended. In all pediatric patients with CAH, particularly minimally virilized girls (Prader I–II) and mildly undervirilized boys (EMS 7–11) (32), parents must be informed about surgical options, including delaying surgery and observation until the child is older.

Usually, the sex assignment in 46,XX newborns with CYP21A2-D or CYP11B1 is female, and genital surgery may be necessary, but the timing of the surgery remains controversial. In patients for whom early surgery is selected, vaginoplasty using urogenital mobilization is suggested, and if selected, neurovascular-sparing clitoroplasty for severe clitoromegaly is suggested (45). With early management (started <2 years of age), 46,XX patients generally have a satisfactory psychosocial outcome (77–79).

In male newborns with severe hypospadias, urological surgery is certainly indicated for functional repair (80). Although a recent review (81) found that 80% of men are satisfied with childhood hypospadias repair, it is advisable to refrain from invasive surgery that is not essential for health and to encourage patient participation and decisions in the choices regarding the sexual sphere (82).

Psychological Support

Diagnosis of classic CAH during neonatal age activates concerns and anxiety in parents related to the risk of electrolyte crises, genital ambiguity at birth, and the effects of hyperandrogenism on the brain, gender behavior, and body perception. The option of genital surgery, in case of highly virilized genitalia, represents a strong stress factor for families. Although studies in the literature show controversial results regarding the quality of life of people with CAH, case reports show that they can be psychosocial consequences related to the ambiguity of the genitalia (impaired bodily self-image, stigmatization, etc.). Therefore, we believe that psychological support is a useful complement to endocrinological and surgical management, in agreement with the Endocrine Society guidelines (45).

AUTHOR CONTRIBUTIONS

AB and FB: conceptualization, methodology, software, CYP11A1-D, CYP21A2-D, and writing—review and editing. AC: resources. AB, FB, and RO: data curation. AB: writing—original draft preparation. RO: StAR-D and CYP17A1-D. AC and VD: 3β-HSD2D and CYP11B1-D. FB, LB, and SM: POR-D. AB and AC: supervision. All authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Clinical Aspects of Neonatal Hypoglycemia: A Mini Review

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Introduction: Neonatal hypoglycemia is common and a preventable cause of brain damage. The goal of management is to prevent or minimize brain injury. The purpose of this mini review is to summarize recent advances and current thinking around clinical aspects of transient neonatal hypoglycemia.

Results: The groups of babies at highest risk of hypoglycemia are well defined. However, the optimal frequency and duration of screening for hypoglycemia, as well as the threshold at which treatment would prevent brain injury, remains uncertain. Continuous interstitial glucose monitoring in a research setting provides useful information about glycemic control, including the duration, frequency, and severity of hypoglycemia. However, it remains unknown whether continuous monitoring is associated with clinical benefits or harms. Oral dextrose gel is increasingly being recommended as a first-line treatment for neonatal hypoglycemia. There is some evidence that even transient and clinically undetected episodes of neonatal hypoglycemia are associated with adverse sequelae, suggesting that prophylaxis should also be considered. Mild transient hypoglycemia is not associated with neurodevelopmental impairment at preschool ages, but is associated with low visual motor and executive function, and with neurodevelopmental impairment and poor literacy and mathematics achievement in later childhood.

Conclusion: Our current management of neonatal hypoglycemia lacks a reliable evidence base. Randomized trials are required to assess the effects of different prophylactic and treatment strategies, but need to be adequately powered to assess outcomes at least to school age.

Keywords: newborn, insulin, screening, diagnosis, continuous glucose monitoring, oral dextrose gel, child development

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INTRODUCTION

Neonatal hypoglycemia is a preventable cause of brain injury. It is common, affecting 5–15% of all babies (1) and approximately half of at-risk babies (2) and is associated with a range of adverse sequelae (3, 4). However, the optimal frequency and duration of screening, as well as the threshold at which treatment would prevent brain injury, remains uncertain. The purpose of this review is to summarize the recent advances in clinical aspects of transient neonatal hypoglycemia.

PATHOPHYSIOLOGY OF NEONATAL HYPOGLYCEMIA

Glucose is the primary metabolic fuel for the fetus. The fetus receives glucose from its mother through carrier-mediated diffusion down a concentration gradient across the placenta (5, 6). Fetal glucose concentrations are ∼80% of maternal concentrations and fluctuate with changes in maternal glucose concentrations (7). The function of insulin in the fetus is as a growth hormone rather than to regulate glucose concentrations, and secretion of insulin occurs at a lower glucose concentration in the fetus than in postnatal life (8).

Maternal and therefore fetal glucose concentrations increase during labor and delivery in response to secretion of maternal stress hormones such as catecholamines and glucocorticoids (9). Once the umbilical cord is clamped, glucose supply is interrupted and neonatal glucose concentrations decrease, reaching a low point ∼1–2 h after birth. In turn, insulin secretion decreases while secretion of counter-regulatory hormones such as glucagon and catecholamines increases, stimulating gluconeogenesis and glycogenolysis, and resulting in a gradual increase in glucose concentrations (9). However, these do not reach adult concentrations until after 72 h of age (10, 11). Delay or interruption of this postnatal metabolic adaptation results in neonatal hypoglycemia.

Glucose is an essential metabolic fuel for the brain, and in the newborn the proportionately large brain accounts for almost all of total tissue glucose requirements (12). Thus, low glucose concentrations are likely to result in inadequate brain energy supply. Although the newborn brain can use alternative metabolic substrates, the supply of these is limited. Lactate provides a potential alternative fuel in the first 48 h, and ketones may be available on days 3–4, but each can provide only a small proportion of total brain energy requirements (13).

DEFINING NEONATAL HYPOGLYCEMIA

The definition of neonatal hypoglycemia remains controversial, and has changed over time (14). However, since the major reason for defining hypoglycemia is to identify a threshold at which treatment would prevent brain injury, an ideal definition would relate to the glucose concentration at which brain function is compromised. This makes a single definition problematic, as the threshold is likely to vary in different babies, depending amongst other things on gestational age, postnatal age, concurrent metabolic demands, co-morbidities and availability of alternative metabolic fuels.

The most widely used definition for neonatal hypoglycemia is a glucose concentration of <47 mg/dl (2.6 mmol/l) (15–17). This arises primarily from two studies published in 1988, which related glucose concentrations to neurological function. One was a retrospective study of 661 preterm babies (birthweight < 1,850 g), which reported that a glucose concentration of <47 mg/dl (2.6 mmol/l) on three or more days was associated with an increased risk of developmental delay at 18 months' corrected age (18). Follow-up of a subgroup showed that reduced motor and arithmetic functioning persisted at 8 years (19).

The second study recorded brainstem or somatosensory evoked potentials in 17 infants, of whom only five were newborns (20). None showed flattening of evoked potentials with a glucose concentration of >47 mg/dl (2.6 mmol/l), although some with a glucose concentration below this still had normal evoked potentials. Both studies concluded that a glucose concentration of >47 mg/dl (2.6 mmol/l) was likely to be safe.

In situations where evidence-based decisions are not possible, operational thresholds offer a pragmatic guide to clinicians for when intervention may be warranted (1). Screening protocols have recommended different operational thresholds ranging from 18 to 60 mg/dl (1.0–3.3 mmol/l) (21–24). However, most recommend aiming for a minimum glucose concentration close to 47 mg/dl (2.6 mmol/l) in late preterm and term babies more than a few hours old or requiring treatment.

TABLE 1 | Risk factors for neonatal hypoglycemia.

Transient neonatal hypoglycemia
Preterm birth
Small or large for dates
Infant of diabetic mother
Perinatal stress (birth asphyxia, hypothermia, respiratory distress, sepsis)
Birth asphyxia
Poor feeding
Maternal use of beta blockers
Antenatal corticosteroids
Persistent neonatal hypoglycemia*
Congenital hyperinsulinism
Hypopituitarism (ACTH deficiency, growth hormone deficiency)
Cortisol deficiency
Glycogen storage disease
Disorders of gluconeogenesis (FBP deficiency, PEPCK deficiency, PC deficiency)
Fatty acid oxidation defects

Occurring after or persisting for ≥3 days (27).
ACTH, Adrenocorticotropic hormone; FBP, Fructose-1,6-bisphophatase; PEPCK, Phosphoenolpyruvate carboxykinase; PC, Pyruvate carboxylase.

INCIDENCE AND RISK FACTORS

The incidence of neonatal hypoglycemia varies between studies depending on the diagnostic threshold, the glucose screening protocol and measurement method used, and the population studied (25). However, the incidence of transient neonatal hypoglycemia is estimated to be 5–15% of newborns (1, 26), and in at-risk babies, it approximates 50% (2) (**Table 1**). Babies with multiple risk factors do not have a higher incidence but may experience more severe hypoglycemia.

MANAGEMENT OF NEONATAL HYPOGLYCEMIA

Screening for Neonatal Hypoglycemia

The clinical signs of neonatal hypoglycemia include, but are not limited to, cyanosis, apnea, altered level of consciousness, seizures, lethargy, and poor feeding (24). However, since many of these signs are non-specific, and the majority of babies with low glucose concentrations show no clinical signs, it is recommended that all babies with risk factors undergo regular glucose monitoring.

The optimal frequency and duration of screening remain uncertain. Most protocols recommend screening within 1–4 h after birth and then every 3 or 4 h until euglycemia is maintained over two or three consecutive glucose measurements (15, 21, 22, 24). However, all of these guidelines are informed by expert opinion and lack a reliable evidence base (28).

Some specify different monitoring periods dependent on the clinical profile of the baby. For example, the American Academy of Pediatrics recommends that monitoring continues until 12 h after birth for infants of diabetic mothers or large for gestational age, but for 24 h for babies who are born late preterm or small for gestational age (21). However, there is no evidence to suggest that cerebral glucose requirements vary between at-risk groups (15).

One study that screened at-risk babies using an accurate glucose oxidase method 1–2 h after birth then every 3–4 h before feeds for the first 24 h and every 3–8 h from 24 to 48 h reported no difference between risk groups in the incidence or severity of neonatal hypoglycemia, suggesting that a single screening protocol would be reasonable for all babies at risk (2).

Blood Glucose Monitoring

Intermittent Glucose Monitoring

A common method for measuring glucose concentrations in neonates is by heel-prick blood sampling analyzed using point-of-care non-enzymatic glucometers. These provide quick results at a low cost, are readily available in neonatal units, user-friendly and require small volumes of blood (29).

However, these devices are designed for monitoring high glucose concentrations in diabetics, and are affected by several factors that vary widely in newborns including bilirubin concentrations and hematocrit. They are inaccurate at low glucose concentrations, with estimated false positive and false negative rates of 10–30%, and are not recommended as the sole method for diagnosis of neonatal hypoglycemia (21, 30). If point-of-care non-enzymatic glucometers are used for screening, it is critical to confirm the results with a laboratory method (21), but best practice is to use more accurate methods from the start.

Laboratory methods use enzymatic reactions including glucose oxidase, hexokinase or dehydrogenase (29) which are more accurate and sensitive for detecting neonatal hypoglycemia (31, 32). However, laboratory methods are costly, take time which can delay prompt intervention, and accuracy is also reliant on the quality of the plasma sample (29). More recent guidelines recommend blood gas analyzers which are quick and accurate if they are immediately available (15, 24).

A more feasible alternative in many settings is the newer enzymatic point-of-care analyzers, which have the same accuracy as laboratory methods but the convenience and speed of a cot-side measurement. Although they are more expensive per test than the widely used (but inaccurate) test strip glucometers, a recent cost analysis concluded that enzymatic glucometers incurred lower direct costs overall because they avoided the additional costs of retesting in the laboratory (33).

Continuous Interstitial Glucose Monitoring

Continuous interstitial glucose monitors comprise a sensor placed under the skin, and a recording device, often remote from the sensor, which converts the electrical current generated in the sensor to a glucose concentration using an inbuilt algorithm. Most devices provide a reading every 5 min, giving detailed information about glycemic control including the duration, frequency, and severity of hypoglycemia (34).

Continuous glucose monitors have several limitations. They require calibration against blood glucose concentrations at least every 12 h, so they do not abolish the need for blood tests, and more frequent calibration is recommended for greater accuracy and precision (35). Continuous glucose monitors are also prone to measurement error, and the reading can drift from the calibrated value without detection (35). Because, like point-of-care glucometers, they are designed for use in diabetes, they are less accurate at low glucose concentrations. The lag period between changes in blood glucose concentrations and changes in the continuous monitor reading is unknown but could be up to 30 min or more, due both to the time required for glucose to diffuse from blood to interstitial fluid, and to delays built into the algorithms, so that the rapid changes in glucose concentrations that are common in newborn babies are poorly reported by continuous monitors (36, 37). Infection at the site of sensor insertion is a theoretical concern, but in practice has rarely been reported, and most studies have reported that sensors can be left in place for a week without complications, even in very low birthweight babies (38).

Most importantly, there is a lack of evidence on whether continuous glucose monitoring is associated with clinical benefits or harms. Continuous glucose monitoring detects many more episodes of low glucose concentrations than does intermittent blood glucose measurement. For example, in 102 babies at risk of hypoglycemia, continuous glucose monitoring identified 11% more babies and 50% more episodes of low glucose than intermittent glucose monitoring (39). Others have reported similar differences (38, 40). Thus, there is a risk that continuous glucose monitoring may lead to a large increase in diagnosis and treatment, but without evidence that these additional detected episodes are related to brain injury, or that additional treatment will have any long-term benefit.

Despite these limitations, continuous glucose monitoring has enormous potential to improve the management of neonatal hypoglycemia. A randomized trial in 48 very low birthweight babies showed that use of continuous glucose monitoring reduced the number of blood samples taken, detected more episodes of neonatal hypoglycemia and reduced the duration of an episode by half when compared with intermittent

glucose monitoring (40). Another randomized trial in 50 very preterm babes reported that continuous glucose monitoring in conjunction with an algorithm for glucose infusion titration reduced the duration and severity of hypoglycemic episodes, thereby promoting glycemic stability (41). However, it is not yet known if this improved stability will lead to improved later outcomes.

Treating Neonatal Hypoglycemia

The goal of treating neonatal hypoglycemia is to prevent or minimize brain injury by maintaining a glucose concentration above an acceptable threshold (25). The usual initial approach is to feed the baby, using either formula or breast milk. When glucose concentrations are <18–25 mg/dl (1.0–1.4 mmol/l) intravenous dextrose (bolus 200 mg/kg followed by an infusion of around 4–8 mg/kg per minute) is usually required (21, 24). However, administering intravenous dextrose involves admission to the neonatal intensive care unit (NICU), which is costly, invasive, and separates the mother from her baby, which in turn can increase maternal anxiety and interfere with the establishment of breastfeeding. Severe or prolonged hypoglycemia, indicated by persistently high or ongoing (≥3 days) intravenous glucose requirements, suggest underlying endocrine or metabolic pathology and further investigation is required (**Table 1**). Elevated insulin concentrations indicate hyperinsulinism, which suppresses the production of alternative metabolic fuels, and hence maintaining blood glucose ≥ 3.5 mmol/l is recommended (24). Additional treatments, such as diazoxide (42), glucagon (24, 43) or glucocorticoids (44) may be required.

Oral dextrose gel 200 mg/kg (0.5 ml/kg of 40% dextrose), in combination with feeding, is increasingly recommended as a first-line treatment for asymptomatic neonatal hypoglycemia (45, 46). A randomized trial of 237 late preterm and term babies at risk of neonatal hypoglycemia [<47 mg/dl (2.6 mmol/l)] demonstrated that compared with feeding alone, 40% oral dextrose gel 200 mg/kg plus feeding resulted in fewer treatment failures (hypoglycemia after two treatment attempts), reduced admission to NICU for hypoglycemia and reduced formula feeding at 2 weeks of age (47). A 2-year follow-up established safety by demonstrating similar rates of processing difficulty and neurosensory impairment between the oral dextrose and placebo groups (48). A subsequent cost-utility analysis concluded that dextrose gel resulted in a cost-saving of US$782 per baby (49).

The incorporation of oral dextrose gel into clinical practice has been evaluated in pre-and post-introduction observational studies in several parts of the world, with most reporting that oral dextrose was associated with a reduced NICU admission and increased breastfeeding (50–54). Its use is now recommended in several national guidelines (15, 22, 24).

Prophylaxis

There is some evidence that even transient and undetected episodes of neonatal hypoglycemia may be associated with adverse sequelae. One study of 1,395 babies born in a center where glucose screening was universal showed that a single episode of transient neonatal hypoglycemia [<35 mg/dl (1.9 mmol/l)] was associated with lower 4th-grade literacy and numeracy proficiency at 10 years of age (55). The Children With Hypoglycemia and Their Later Development (CHYLD) study demonstrated that clinically undetected low interstitial glucose concentrations were associated with an increased risk of executive dysfunction at 4.5 years of age (56). These findings suggest that even an effective treatment for neonatal hypoglycemia would not be sufficient to optimize outcomes for all babies, and prophylaxis needs to be considered.

The prophylactic measures currently recommended include early feeding, ensuring babies are warm and dry, and early skin-to-skin contact (57). These measures are thought to have a glucose sparing effect (58), but the evidence that they alter blood glucose concentrations or the incidence of hypoglycemia is limited (59–61).

Oral dextrose gel is being tested as an additional prophylactic measure to prevent hypoglycemia in at-risk babies. A dose-finding trial (Pre-hPOD) of 416 at-risk babies randomized to either placebo or dextrose gel at one of four different dosing schedules reported that a single dose of prophylactic oral 40% dextrose gel (200 mg/kg) in combination with breastfeeding was the most effective and practical dose (62), with a number needed to treat to prevent one case of hypoglycemia of 10. Further, the treatment was found to be acceptable, well tolerated, and had no adverse events (62). Follow-up at 2 years' corrected age showed no adverse effects, similar rates of neurosensory impairment between the groups, and a trend toward improved executive function scores in the dextrose gel group (63).

A quasi-experimental study of 236 at-risk babies reported that compared with feeding, prophylactic oral dextrose gel 200 mg/kg was not associated with a decreased incidence of hypoglycemia [<40 mg/dl (2.2 mmol/l)] or admission to NICU (64). However, this study was not randomized, and the preparation used (Insta-Glucose gel) includes additional carbohydrates other than dextrose, which are likely to have competed with dextrose for membrane uptake and potentially reduced the effectiveness of this approach.

A multicenter randomized trial (hPOD) investigating whether prophylactic oral dextrose gel prevents neonatal hypoglycemia and hence reduces NICU admission has finished recruitment (ANZC Trials Registry – ACTRN12614001263684) (65). The results, and particularly the findings of the planned long-term follow-up, will provide valuable insight into whether prophylaxis with dextrose gel should be introduced into clinical practice.

OUTCOMES OF NEONATAL HYPOGLYCEMIA

Magnetic Resonance Imaging (MRI) studies have shown that neonatal hypoglycemia can cause brain injury (66, 67). The most widely reported pattern of acute brain injury is localized in the parietal and occipital regions (68), which are involved in visual processing. However, the evidence is inconsistent on whether neonatal hypoglycemia is associated with later visual problems (69). Injury may extend beyond these regions with reports of

global or periventricular damage (67) as well as damage to the basal ganglia and thalamic regions (67, 70).

A systematic review and meta-analysis of six cohort studies with a sample size of 1,675 babies reported that neonatal hypoglycemia [definitions ranged from <20–47 mg/dl (1.1–2.6 mmol/l)] was not associated with neurodevelopmental impairment, cognitive or motor deficits between 2 and 5 years of age (4). However, neonatal hypoglycemia was associated with a 3-fold increased risk of visual-motor impairment and executive dysfunction at 4 years of age. These risks were heightened for children who had experienced severe, recurrent or clinically undetected neonatal hypoglycemia (56). In older children, limited data (two studies, sample size of 54 babies) showed that neonatal hypoglycemia was associated with more than a 3-fold increased risk of neurodevelopmental impairment at 6–11 years of age, and a 2-fold increase in low numeracy and literacy (4). No studies reported on outcomes for adolescents.

Most of the evidence about long-term outcomes after neonatal hypoglycemia comes from retrospective observational studies, few of which have controlled for potential confounders or looked at outcomes beyond very early childhood. For example, infants of mothers with diabetes, who are at increased risk of neonatal hypoglycemia, have an increased risk of adverse outcomes (71, 72), but it is unclear how much of this risk is attributable to neonatal hypoglycemia. There is high heterogeneity between the studies which made comparing outcomes problematic, and there have been frequent calls for robust randomized trial evidence (3).

A randomized non-inferiority trial was the first to begin to address this major knowledge gap by comparing treatment at a threshold of 47 mg/dl (2.6 mmol/l) against treatment at a lower threshold of 36 mg/dl (2.0 mmol/l) among a sample of 689 otherwise healthy late preterm and term babies with mild-moderate hypoglycemia [36 mg/dl (2.0 mmol/l)−46 mg/dl (2.5 mmol/l)] (73). Babies with early (birth to 2 h) and severe [≤35 mg/dl (1.9 mmol/l)] hypoglycemia were excluded. In babies randomized to treatment at the lower threshold, fewer were monitored and treated, but there were more severe and recurrent hypoglycemic episodes (≥4 episodes) compared with babies in the higher threshold group. Hospital costs and duration of stay were similar between the groups, as were motor and cognitive functioning at 18 months on the Bayley Scales for Infant and Toddler Development. However, since previous studies have shown no relationship between neonatal hypoglycemia and motor or cognitive function at this age (4), this finding is not surprising, and the greater exposure to severe and recurrent hypoglycemia in the low threshold group is of concern. Much longer follow-up, at least to school age, will be essential to realize the true value of this important study.

CONCLUSION

Over the last few years, neonatal hypoglycemia has received much attention. However, what remains unclear is the extent to which transient asymptomatic neonatal hypoglycemia is associated with brain injury and neurodevelopmental impairment, and if so, at what glucose concentration maintained for how long. To address this, adequately powered randomized trials are needed of both prophylactic and treatment interventions at different glucose thresholds, with neurodevelopmental outcomes assessed at least to school age.

AUTHOR CONTRIBUTIONS

TE and JH contributed to the literature search and the design of the review. TE wrote the first draft of the manuscript and contributed to further revisions. JH contributed to the writing of the manuscript and supervised the study. Both authors contributed to the article and approved the submitted version.

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MINI REVIEW
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Minipuberty: Looking Back to Understand Moving Forward

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Hypothalamic-pituitary-gonadal (HPG) axis activation occurs three times in life: the first is during fetal life, and has a crucial role in sex determination, the second time is during the first postnatal months of life, and the third is with the onset of puberty. These windows of activation recall the three windows of the "Developmental Origin of Health and Disease" (DOHaD) paradigm and may play a substantial role in several aspects of human development, such as growth, behavior, and neurodevelopment. From the second trimester of pregnancy there is a peak in gonadotropin levels, followed by a decrease toward term and complete suppression at birth. This is due to the negative feedback of placental estrogens. Studies have shown that in this prenatal HPG axis activation, gonadotropin levels display a sex-related pattern which plays a crucial role in sex differentiation of internal and external genitalia. Soon after birth, there is a new increase in LH, FSH, and sex hormone concentrations, both in males and females, due to HPG re-activation. This postnatal activation is known as "minipuberty." The HPG axis activity in infancy demonstrates a pulsatile pattern with hormone levels similar to those of true puberty. We review the studies on the changes of these hormones in infancy and their influence on several aspects of future development, from linear growth to fertility and neurobehavior.

Keywords: minipuberty, neurobehavior, neonate, gonadotrophins, hypothalamic-pituitary-gonadal axis, hypogonadism

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INTRODUCTION

During embryogenesis, the pituitary gland begins synthesizing both Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH) at around 9 weeks of gestation (1). LH and FSH can be detected in fetal blood from 12 to 14 weeks (2, 3) and start to be GnRH-dependent after 31–32 weeks (4, 5).

Prenatal modulation of the HPG axis activity is also due to placental hormone production. In fact, the structure of hCG is an analog of LH and may bind to the LH receptor, with similar biological effects on gonadal tissues (2, 6). Moreover, the placenta produces Estrogens (E) and Progesterone (P), that rise during the third trimester. This has a negative effect on gonadotropin levels and results in a drop in LH and FSH in cord blood at birth in healthy infants of both sexes (6–8).

After birth, the removal of placental hormones from the neonate's circulation results in a lack of negative feedback on the GnRH pulse generator and reactivation of the HPG axis. This postnatal activation that starts in the first few days of life is known as "minipuberty" (9).

Studies on healthy term neonates indicate that the rise of LH and FSH begins at around 1 week of age. It achieves a peak, reaching the pubertal range, between 1 and 3 months of life and then declines toward the age of 6 months (6–8, 10–13). These postnatal hormonal changes have different trends in boys and girls. Particularly in males, it seems to be related to the development and maturation of the reproductive system. Furthermore, the impact of gonadotropins and sex-steroid hormones during this first period of life has been studied and relates to many different aspects of infant growth and behavior (14).

The aim of this review is to summarize the current understanding on minipuberty and its role as a temporary window of opportunity for diagnosis and possible treatment in babies with disorders of sex development (DSD). Moreover, we would like to highlight the extent of what happens (or not) during minipuberty in terms of hormonal changes and trends which may influence future neurobehavior.

INFANTS BORN AT TERM

Males: In male neonates, both LH and FSH levels peak between 1 and 3 months of age and then gradually decrease to prepubertal levels at around 6-9 months (9, 12, 14). The LH peak is higher than the FSH level. Testosterone (T) starts to increase 1 week following the LH rise and declines to prepubertal values by 6 months of age (11, 12, 15). T levels, both in cord blood and in serum during the first postnatal months, are higher in boys than in girls (11–13, 15–17).

The number of Leydig cells in both testes increases considerably until the third month of life, which correlates with the T trend and then gradually decreases due to an apoptosis process (18, 19). Sertoli cells also grow during the first postnatal months under the stimulation of FSH (20) but, without the expression of androgen receptors (AR) during infancy, they do not complete their maturation and spermatogenesis does not occur (21). This leads to an increase of testicular volume during the first months after birth, which then gradually decreases until the second year of life due to the halt in cell proliferation, the reduction of AMH production, and the formation of the blood-testicular barrier (22, 23).

All these hormonal changes during the first months of life have a great impact on the urogenital system. This involves not only the testes but also the development and growth of the penis, prostate, and scrotal hair. In fact, the postnatal T surge within the first three months has been associated with penile growth in infancy (24). The increase in androgens has been associated with cutaneous manifestations, such as sebaceous gland hypertrophy and acne (25). There is also a link with the development of transient isolated scrotal hair between 3 and 6 months of life with a spontaneous disappearance within the first year of life (26).

Females: In female infants, FSH levels are higher than LH, following a different trend than in males. FSH shows the same gonadotropin peak as in males at 1–3 months of age but can remain elevated up to 3–4 years of life. In contrast, LH levels decrease at the same age as in boys (9, 12, 27).

E levels at birth are high, with similar values in the cord blood of both sexes (28), followed by a gradual decrease during the first days of life and a new increase after the first week only in girls.

E remains high until 6 months with fluctuating levels, probably related to the FSH trend, and decreases toward 2 years of life (29–31). The mammary glands and uterus are certainly E target tissues but evidence of minipuberty effects is not univocal. At birth, most full-term babies of both sexes have palpable breast tissue (32) that probably results from placental E effect. In the following months, breast tissue in females remains larger and persists longer due to HPG axis activity and its consequent E production (30). In contrast, uterine length increases *in utero* but, after birth, there is a steady decrease from day 7 toward the third month, after which the volume remains stable until the second year (30).

With little evidence from few studies, the biological role of minipuberty in girls is still controversial and partially unknown.

Babies **born small for gestational age (SGA):** The HPG axis activation in SGA infants born at term is not well defined and its short-term and long-term effects on growth and development are still controversial. Studies on SGA females found higher postnatal FSH levels compared with neonates born appropriate for gestational age (AGA). This different pattern of secretion in SGA females was also associated with reduced uterine and ovarian size that persisted into young adulthood (33, 34). Moreover, Anti Mullerian Hormone (AMH) levels have been reported to be higher in SGA girls at 2–3 months of life, suggesting possible altered follicular development (35).

In contrast with these findings, other studies have reported higher E in SGA females after the administration of a GnRH stimulation test, although the reported basal levels were not significantly different (35).

In male SGA term neonates, HPG axis activation has been linked both to lower (36) and higher (34) FSH and T (37) levels, with uncertain effects in adult life (38).

Further studies are necessary to clarify the pattern of minipuberty in SGA male and female infants, along with the clinical implications. It is important to bear in mind that SGA neonates are at increased risk of metabolic and endocrinological disorders. These include reduced insulin sensitivity and increased adrenal hyperandrogenism, with consequent precocious pubarche and reduced ovulation rate (39).

PRETERM INFANTS

Little is known about the influence of prematurity on HPG axis activity and its effects. Fewer studies have investigated this pattern longitudinally in preterm (PT) babies compared with those born full-term (FT). Preterm birth does not seem to influence the postnatal HPG axis activation, as gonadotropin levels begin to rise after birth (whenever that is, as fetal-placental interruption) with the same timing as in FT infants.

Moreover, this hormonal surge might be even stronger and more prolonged than in FT infants (40, 41). However, these data are not univocal (40–42) in either the amplitude or the duration between different sexes. Immaturity of the

hypothalamic feedback has been suggested as a possible mechanism for this strong and prolonged activation, although its biological significance is still not completely understood.

The most recent longitudinal data suggest that minipuberty declines at about the same post-term age in term neonates compared with premature infants, suggesting that the HPG activity is regulated in an evolutionarily way (13). In particular, Kuiri-Hanninen et al. (13) used spot urine samples in order to compare gonadotropin and testosterone levels in a small cohort of FT and PT male neonates with a gestational age (GA) between 24.7 and 36.6 weeks. From day 7 to 14 months of age they measured length, weight, penile length, and testicular volume. They simultaneously collected urine samples to detect urinary gonadotropins and testosterone levels until 6 months of age. Their findings revealed higher hormonal levels in PT babies with a positive association between testosterone levels and penile growth, as well as between FSH levels and testicular growth after birth until 5 months of age, when a subsequent decrease occurred. In addition, studies on PT female infants demonstrated higher gonadotropin levels than those in FT girls with a prolonged duration of the peak (43) but a sharp decrease around term age (30). In these PT girls, an amplified postnatal E surge was observed at around three months of corrected age and there was an association with increased growth of the mammary gland and uterine length. Possible clinical consequences of this intensive stimulation in premature females are evidenced by features of the ovarian hyperstimulation syndrome with edema of the vulva, solitary or multiple cysts in the ovaries on ultrasonography, breast growth, and occasional vaginal bleeding (44, 45).

The hormonal differences between boys and girls during minipuberty appear to be fundamental for later sexual differentiation and development. In particular, we think that increasing knowledge on minipuberty in girls may give us key information about the premature thelarche of girls below 2 years of age, and the early puberty that occurs before 8 years of age. In SGA neonates, results are still controversial and more studies are needed to clarify how gonadotrophins and sexual hormones change according to sex. This may be very useful, considering that SGA-born children may go through early puberty and/or precocious isolated pubarche. In preterm babies, we speculated that the prolonged activation of HPG axis may be one of the factors influencing the early re-activation of the HPG axis before puberty age.

BABIES WITH DISORDERS OF SEX DEVELOPMENT

The development of internal and external genitalia is a complex balance between gene expression and hormonal influence and an anomaly at each stage can result in 46, XY DSD.

From this point of view, minipuberty can be considered as a window of sensitivity, because it may allow the clinician to come to an early diagnosis and possible treatment opportunity.

Studies on primates testing the effects of a reversible suppression of minipuberty using GnRH agonists or antagonists described lower testis volume and penile length in cases treated compared with controls (46–48). Male infants with congenital central hypogonadism (CHH) were found to have an absence of both fetal and postnatal FSH, LH, and T surges (49). This lack of postnatal FSH secretion seems to be the main reason for impaired germ cell differentiation with later infertility, especially if associated with cryptorchidism (50, 51). As a result, minipuberty may potentially provide a short window of time to make an early diagnosis and for treatment in male neonates that exhibit a micropenis with or without cryptorchidism (52, 53), improving the outcome of orchidopexy, and also reducing the long-term consequences of an absent minipuberty.

On the other hand, the finding of elevated gonadotropins during minipuberty in a 46, XY male neonate with undetectable testosterone levels may suggest congenital anorchism (vanishing testis or testicular regression syndrome). Infants with complete androgen insensitivity syndrome may present with lower-than-normal postnatal LH and T levels, whereas these hormones may be normal or high in cases of partial androgen insensitivity syndrome (54).

We have a unique opportunity to evaluate the spontaneous function of the HPG hormone axis during minipuberty. It is therefore recommended that serum FSH, LH, and testosterone are measured during the first 6 months of life in infants with DSD or suspected CHH. In particular, we would suggest checking for these hormones at seven days of life, and one, three, and, if possible, 6 months of life, to detect the minipuberty trend. The use of the LH/FSH ratio may provide important information in the workup of infants suspected of DSD, especially regarding the sex specific ratio detected in literature (55).

MINIPUBERTY: A WINDOW FOR TREATMENT?

Neonates affected by cryptorchidism, micropenis, and CHH must receive timely treatment to optimize genital development. The current recommendation for a micropenis is brief therapy with low-dose testosterone delivered by intramuscular injection or by topical application to induce penile growth. This treatment was also assumed to work for cryptorchidism. However, while exogenous T stimulates penile growth, it does not affect testicular development. In fact, current recommendations advocate surgical correction for undescended testes during the first year of life (56). Nevertheless, there are limitations to this treatment. Small testes augment the risk of testicular trauma and tissue loss during orchidopexy (57), increasing the likelihood of a negative impact on future fertility. Moreover, successful scrotal repositioning of testes does not prevent infertility. However, a normal minipuberty after successful surgery may lead to the presence of Ad spermatogonia (58). The role of Ad spermatogonia is to maintain the supply of stem cells for spermatogenesis. In 178 testicular biopsies after orchidopexy the authors found three groups of high, intermediate, and low risk of infertility depending on the presence of Ad spermatogonia. After puberty, sperm concentrations were analyzed and correlated positively with plasma gonadotropin and testosterone levels. For all these reasons, recreating the hormonal milieu of

minipuberty with gonadotrophin treatment could be beneficial for these patients.

In 2002, Main et al. (59) published the first case of CHH and micropenis treated with short-term recombinant human LH and FSH. The outcome of this case was successful; the penile length increased by 50% and the testicular volume almost tripled. Similar results were described in other recent cases (60, 61).

The REMAP study (62) investigated the use of recombinant LH plus FSH preparations in neonates and infants with a micropenis and/or cryptorchidism due to hypogonadotropic hypogonadism. During therapy, all ten patients increased their height velocity: LH levels increased from undetectable to high-normal; FSH reached supranormal levels; and Inibin-b, AMH, and T reached normal levels. Penile length normalized among all children and intriguingly confirms the emerging evidence that testicular descendance is induced by gonadotrophin treatment (61, 63). Furthermore, in this study the therapy may have induced high/normal activation of Sertoli and Leydig cells, restoring testicular endocrine function and improving future fertility.

Vincel et al. (64) analyzed testicular biopsies before and after orchidopexy or hormonal treatment in patients with isolated bilateral cryptorchidism with a high infertility risk. Their results showed how the number of Ad spermatogonia and the number of germ cells per at least 100 tubular cross-sections increased or decreased post-surgery. Indeed, patients who received hormonal treatment showed an important increase in the number of cells and the complete transition of gonocyte and fetal spermatogonia to Ad spermatogonia. These findings support the hypothesis that GnRH induces LH release; LH increases testosterone levels acting directly on Leyding cells, mimicking minipuberty (50, 65).

Finally, studies have focused on the molecular mechanisms that explain the ability of GnRH to rescue fertility. The analysis demonstrates that several IncRNAs involved in epigenetic programming were responsive to GnRH treatment, helping in the preparation of Ad spermatogonial stem cells for commitment to differentiation. In particular, the authors found that DMRTC2, PAX7, BRACHYURY/T, and TERT were associated with defective minipuberty and were responsive to GnRHa (66). Minipuberty may represent a "window of opportunity" to evaluate the HPG axis by measuring basal hormone concentrations with no need for stimulation tests in infants with suspected reproductive disorders. Minipuberty provides a unique opportunity to evaluate the spontaneous function of the HPG axis which is lost thereafter for approximately another 10 years until the HPG axis is reactivated in puberty (67).

IS OUR ENVIRONMENT INFLUENCING MINIPUBERTY IN HUMANS AND PREDISPOSING THEM TO DSD?

Endocrine Disruptor Chemicals (EDC) are compounds detectable in every setting of daily life. These chemical compounds are found in a range of products such as those containing pesticides, metals, additives or food contaminants, and personal care products (68). In fact, EDCs are so common that it is almost impossible for individuals to avoid them during everyday activities. These substances may cause adverse health effects, disrupting endocrine function. In particular, they interfere with the endocrine and reproductive systems through nuclear receptors, non-nuclear steroid hormone receptors, non-steroid receptors, orphan receptors, enzymatic pathways, and other mechanisms (69). Children may be exposed both directly and indirectly to ECDs, especially during the three main temporal windows of the DOHaD paradigm and during breastfeeding (70, 71). Concerning breastfed children, Ortega-Garcia et al. detected a linear positive correlation between anogenital distance (AGD) in male infants and the duration of breastfeeding (72). The results of this study, called MALAMA, suggested breastfeeding to be a protective factor against the reduction of the AGD of 2-year-old boys. The authors hypothesized this could be related to early exposure to EDCs through baby formula milk (72).

Moreover, EDCs may interfere with HPG activation (73), both during fetal life or immediately after birth, throughout minipuberty. EDCs during minipuberty in males could impair testicular descent (74). Focusing on some of the most analyzed compounds in this research area, several studies have demonstrated that Bisphenol A (BPA) has an anti-androgen function, decreasing testosterone levels, an event that impacts sex differentiation during fetal life and modifies the AGD length (75, 76). In particular, Sun et al. showed how maternal exposure to BPA was associated with shortened AGD in boys at 12 months of age, highlighting a gender specific effect (77). Another family of EDCs influencing minipuberty are phthalates. Maternal exposure to phthalates during pregnancy showed a reduced T level in males at minipuberty and, because of the antiandrogenic effect of these compounds, the testosterone-luteinizing hormone ratio (T/LH) is also lower in the same period (78). This is probably due to compensated Leydig cell function, requiring higher levels of LH to maintain the necessary level of T for embryo differentiation. It was demonstrated in animal models that phthalates can inhibit Insl3 production and consequently modify the gubernaculum growth necessary for the testes' transabdominal descent (79, 80). However, the effects of these compounds on Insl3 and T in the human testes were less attenuated than in rodents. **Table 1** summarizes the most recent studies on the effect of EDC on HPG axis during minipuberty in humans. The impact of EDCs is not limited to the postnatal period. Indeed, alongside this phase there are two more windows of development: fetal and puberty. In these phases, cells are promptly proliferating, and epigenetic changes are more likely to occur (81). All this may lead to additional effects in later stages of life including delayed or precocious puberty (82–85), small testes and high levels of follicle-stimulating hormone (FSH) (86, 87), polycystic ovary syndrome (88), and breast cancer (89).

Our environment plays a crucial role in developmental programming. The influence of EDCs on minipuberty may predispose an individual to undescended testis, AGD modifications, or reduction of T surge. We should always keep an eye on the appearance of the external genitalia in neonates

TABLE 1 | Possible effects in humans of the main EDCs on minipuberty and long-term consequences (68–89).

EDcs	Possible mechanism of action	Effects on minipuberty	Future effects
BPA	Increased estrogen receptor Inhibition of apoptotic activity in breast tissue	Lower T level Shortened AGD in boys	Premature thelarche Breast neoplastic transformation Infertility
Phthalates	Reduced T synthesis Modified estrogen activity Antiandrogenic effect Insl3 inhibition	Lower T/LH ratio Undescended testis Hypospadias Shortened AGD	Early puberty Premature thelarche Delayed pubic hairs development Increased breast cells proliferation Less recruitment of primary follicles PCOS Spermatogenic failure and infertility
PBDEs/PBB	Modified estrogen activity Antiandrogenic effect	Undescended testis	Early pubic hair stage (boys) Early/late menarche in breastfed girls Early puberty Anticipated menarche
DDT/DDE	Modified estrogen expression Antiandrogenic effect	Undescended testis Hypospadias	Precocious puberty Anticipated menarche Later onset of puberty Increased risk of breast cancer Testicular cancer
PCBs	Augmented level of FSH and estradiol Antiandrogenic effect	Undescended testis Hypospadias	Anticipated menarche Delayed puberty Augmented adipose tissue in breast Semen alteration

and on the possible maternal exposure to phthalates and BPA through a specific interview.

HOW MINIPUBERTY INFLUENCES LINEAR GROWTH DURING THE FIRST 6 MONTHS OF LIFE

During minipuberty, the transient HPG axis activation results in a sex steroid surge. Some studies have indicated a higher growth velocity and a faster increase in weight (and lean body mass) associated with somatic changes in boys when compared with girls during the first 6 months of life (90–93). Based on these results, studies have tested the hypothesis of an association with minipuberty, particularly with the peak of testosterone production. Kiviranta et al. (94) evaluated the precise timing and the magnitude of this sexual dimorphism in growth among a large cohort of full-term healthy boys and girls during the first years of life. In a smaller sample of healthy neonates, serial measurements of urinary and blood hormones were assessed. Results from this study demonstrated that linear growth was significantly faster in boys than in girls, especially when comparing the first three months of age. Interestingly, this observation occurred simultaneously with the peak of postnatal gonadal activation and the authors found a positive correlation between T levels and growth velocity in both sexes, elucidating a possible novel biological role of minipuberty as an engine of growth velocity during the first months of life. Differences in sex hormones during minipuberty between boys and girls are important for the sex differentiation in linear growth and body composition, with males having a higher growth velocity and accumulating more lean mass compared to females.

HOW MINIPUBERTY MODULATES NEUROBEHAVIORAL DEVELOPMENT

As for sexual development, the human brain is also shaped by a combination of genetic, epigenetic, environmental, and hormonal exposure. Sex steroid hormones are among one of the strongest biological factors influencing neural and behavioral development. Over the past two decades, there has been a growing interest in understanding how sex determination and sexual hormones may affect structural and functional brain development (95, 96). The cellular and molecular mechanisms induced by T (converted to estradiol in the brain) are multifaceted and include neurogenesis, cellular differentiation, axon guidance, synaptic pruning, apoptosis, and phagocytosis. Several studies on mammalian brains, including humans, have demonstrated that early androgen exposure has an influence on sex differences in juvenile behavior (54, 96, 97). Indeed, manipulating androgens prenatally in non-human primates alters brain regions and behaviors (98). Many studies have been performed in girls prenatally exposed to high levels of androgens because of congenital adrenal hyperplasia (CAH) where there is strong evidence of male typical play behavior, suggesting a similar hormonal influence on human brain development (98–104). This influence of androgen levels on the brain has been identified not only among affected girls but also in the general population. Fetal T measured from amniotic fluid positively correlates with male typical play in preschool girls and boys assessed with a standardized questionnaire (105). This prenatal

TABLE 2 | Main studies on minipuberty and neurobehavior.

References	Methods	Results	Future prospectives
Lamminmäki et al. (110)	– **Urinary testosterone** at 7 days of age (D7), and months 1, 2, 3, 4, 5 and 6 (M1–M6) – The **PSAI** is a 24-item, standardized questionnaire designed to discriminate gender related behavior within the sexes, as well as between girls and boys (111). It has been validated in the age-group 2 to 7 years. The questions covers three aspects of behavior: ***play with sex-typed toys*** (e.g., dolls, cars), ***engagement in sex-typed activities*** (e.g., ballgames, playing at cooking/cleaning) ***and sex-typed child characteristics*** (e.g., interest in snakes/spiders/insects, liking pretty things). – **The toy preference test**: the child was seated in the middle of a semi-circle formed by 9 toys. These toys were selected to be ***female- preferred*** (a tea set, a soft doll, and a baby doll and bathtub), ***male-preferred*** (a truck, a train, and a parking structure with two motorcycles), or ***gender neutral*** (a teddy bear, a soft picture book, and a set of keys). Two toys from the same category were never adjacent to each other. The session of 10 min was videotaped and the score consisted on how long (in seconds) that the child played with each toy, with play defined as the child touching the toy for 1 s or longer.	– In boys, **urinary testosterone** concentrations peaked at 1 month postnatal and decreased to low levels by the age 6 months. In girls, urinary testosterone concentrations were slightly elevated at D7 and M1, and then decreased to low levels. In the overall population, urinary testosterone was significantly higher in boys than in girls. – In boys, but not in girls, testosterone AUC correlated positively with **PSAI** scores. – Both boys and girls played significantly more with the same sex-related toy. – Testosterone in boys was negatively related to the female-preferred toy playing, but not in girls. Testosterone in girls was positively correlated with the male-preferred toy playing. A significant negative association between testosterone and time spent playing with the truck and a significant positive association between testosterone and time spent playing with the soft book among boys was detected.	The study underlined how testosterone may exert organizational effects on neurobehavioral development during early infancy both in girls and in boys. The urinary sampling method could be easier to be used in neonates and infants.
Constantinescu et al. (116)	61 healthy infants (29 males, 32 females) and 59 mothers and 3 fathers. **Saliva samples** of **testosterone** when infants were 1–2.5 months of age, and **mental rotation** performance was assessed at 5–6 months of age. Mental rotation ability was assessed using the procedure developed by Constantinescu et al. (116). The stimuli were video representations of dynamic 3D objects, depicted in rotational movement around their vertical axis in 3D space.	Testosterone concentrations were significantly higher in boys than in girls at age 1–2.5 months. In contrast, at age 5–6 months, testosterone concentrations were significantly lower in both sexes than they were at the first visit. – male infants spent a significantly longer time looking at the novel stimulus than at the familiar one, and 65% of the male infants preferred the novel stimulus. – In contrast, female infants looked at the familiar and novel test stimuli about equally, and 46% of the female infants preferred the novel stimulus. These findings suggest that more male than female infants had developed an ability for mental rotation at 5–6 months of age. – The male infants' novelty preference was significantly greater than that of the female infants – Testosterone concentrations at 1–2.5 months of age correlated significantly with novelty preference scores on the 3D mental rotation task in 5- to 6-month-old boys but not in girls.	– Testosterone may have organizational influences on mental rotation performance – In girls, mental rotation performance at age 5–6 months correlated negatively with parents' traditional attitudes on gender. This finding suggests that parents could influence their daughters. "mental rotation abilities" beginning very early in life.

(Continued)

TABLE 2 | Continued

References	Methods	Results	Future prospectives
Kung et al. (114)	– -Saliva samples for testosterone between 1 and 3 months old. – -Between 18 and 30 months, all of the parent participants were invited to complete an online questionnaire assessing the children's expressive vocabulary size – -The toddler short form for vocabulary production from the **MacArthur Communicative Development Inventory [CDI;** (117)] is a parent-report measure designed to assess expressive vocabulary production in toddlers aged 16–30 months	– -boys had significantly higher concentrations of testosterone during mini-puberty and significantly lower CDI scores at age 18–30 months than girls – there was a significant negative correlation between concentrations of testosterone during mini-puberty and later CDI scores in boys – Differences were found between boys and girls in salivary testosterone at 1–3 months of age and in expressive vocabulary size at 18–30 months of age. A negative link between testosterone during mini-puberty and expressive vocabulary was found in boys, in girls, and in the entire sample. Results also showed that testosterone accounted for significant additional variance in expressive vocabulary, when other predictors, such as child's age at vocabulary assessment and paternal education were controlled, suggesting that the effects of testosterone are independent from those of other predictors – higher concentrations of salivary testosterone during the peak of mini-puberty at age 1–3 months predicted smaller expressive vocabulary at age 18–30 months in boys and in girls.	Similar future research might usefully assess the independent contributions of prenatal and postnatal androgen exposure to expressive vocabulary and to other aspects of development that also differ by sex.
Kung et al. (118)	Testosterone in saliva samples collected from children at 1 to 3 months of age (40 boys, 47 girls). When the children reached 18 to 30 months of age, parents completed the **Quantitative Checklist for Autism in Toddlers (Q-CHAT)**.	Boys had higher concentrations of testosterone postnatally and higher Q-CHAT scores than girls. However, testosterone did not correlate with Q-CHAT scores in boys, girls, or the entire sample. There is no relationship between testosterone exposure during mini-puberty and autistic traits.	This does not preclude effects of mini-puberty on other behaviors (see the gender-typed play behavior). Other studies have hypothesized a correlation between prenatal exposure to testosterone and autistic traits.
Tanja Kuiri-Ha nninen et al. (13)	Urinary gonadotropins and testosterone were measured in serial urine samples and compared with testicular and penile growth in preterm (PT) and full term (FT) neonates. Urinary prostate-specific antigen was measured as an androgen biomarker.	LH and testosterone levels were higher in PT boys than FT boys. Compared with FT boys, FSH levels were lower at day 7 but higher from month 1 to month 3 in PT boys. This was associated with significantly faster testicular and penile growth in PT boys compared with FT boys.	Postnatal HPG axis activation in infancy is increased in PT boys and associated with faster testicular and penile growth compared with FT boys. As mentioned in **Table 1**, there is a possible long-term consequence of hyperandrogenism in PT infant boys warrant further research.
Pasterski et al. (113)	– Penile length, Ano-genital-distance (AGD), and body length were among the growth parameters assessed as part of the larger baby growth study. Measurements were taken at birth, and at 3, 12, 18, and 24 months of age in typically developing infants. – Gender-related behavior was measured at 3 to 4 years of age using the Preschool Activities Inventory (PSAI).	AGD at birth and penile growth during the first three months postnatal independently predicted increased masculine/decreased feminine behavior in boys at 3 to 4 years of age. AGD at birth may be employed as a biomarker of prenatal androgen exposure, while penile growth during mini-puberty may reliably reflect variance in early postnatal androgen exposure.	Future research could use these biomarkers in large-scale population studies to further elucidate neurobehavioral effects of perinatal androgen exposure. Such large-scale investigations could also permit a prospective assessment of other factors known to influence variance in gender-related behavior, such as socialization and cognitive development, along with their interactions with early androgen exposure.

period of HPG axis activation is therefore critical for the sexual differentiation that drives the different organization in circuitry and neuroanatomy between the male and female brain. The early postnatal surge of gonadotropins and T in boys during minipuberty can potentially provide a window of opportunity in understanding the effects of sex steroid hormones on human gender development (106). Emerging evidence suggests that T levels during minipuberty have an influence not only on male genitalia and reproductive function but also on later gender-typical behavior. Indeed, minipuberty occurs during a period of huge and rapid brain development in terms of volume, cortical thickness, surface, and cortical network development (107–109). Lamminmäki et al. (110) found a positive correlation between T levels in FT infants from day 7 to 6 months and future sex-typed behavior at 14 months of life. In this study, the Pre-School Activities Inventory (PSAI) (111, 112) playroom was used during an observation of toy choices. In boys, T levels correlated significantly with PSAI scores and playing with trains. Conversely, playing with dolls was significantly correlated with a negative trend. In addition, Pasterski et al. (113) used AGD at birth and penile growth from birth to 3 months of age to estimate prenatal and postnatal androgen exposure. They re-evaluated children included in the study at 3 to 4 years of age using the PSAI suggesting that T levels in both periods, prenatal and postnatal, are independent contributors to later gender-related behavior. Language development is another area of investigation of a possible correlation with early postnatal HPG axis activation. Results based on small samples suggest a correlation between T levels and a different expressive vocabulary in boys and girls (114, 115). We have summarized some of the clearest studies of the last decade in **Table 2** in order to better understand the influence of hormonal changes happening during minipuberty on sex-related behavior. All these emerging results may support a role for the imprinting of T during early infancy in human neurobehavioral sexual differentiation, although its effects are still largely unknown in both the short and long-term.

CONCLUSION

Although further studies are needed, pre- and postnatal activation of the HPG axis could be considered an important window of prediction on how each newborn will grow and develop. Measurement of LH, FSH, and testosterone at 7 days, one and three months, and, when possible, six months, may help the clinician to better understand how minipuberty develops in different neonates. This will give us important information once the baby approaches puberty or when he or she shows impaired linear growth. Moreover, minipuberty must be considered a fundamental moment for possible therapeutic intervention in DSD. Therapeutic interventions may be able to change the natural history of some DSD or, at least, to improve prognosis in terms of fertility and quality of life.

AUTHOR CONTRIBUTIONS

LL, VT, ABo, and LM analyzed the existing literature and wrote the draft of the review. ABe and LI critically reviewed the manuscript. All authors read and approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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REVIEW
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Congenital Hypopituitarism During the Neonatal Period: Epidemiology, Pathogenesis, Therapeutic Options, and Outcome

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Introduction: Congenital hypopituitarism (CH) is characterized by a deficiency of one or more pituitary hormones. The pituitary gland is a central regulator of growth, metabolism, and reproduction. The anterior pituitary produces and secretes growth hormone (GH), adrenocorticotropic hormone, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, and prolactin. The posterior pituitary hormone secretes antidiuretic hormone and oxytocin.

Epidemiology: The incidence is 1 in 4,000–1 in 10,000. The majority of CH cases are sporadic; however, a small number of familial cases have been identified. In the latter, a molecular basis has frequently been identified. Between 80–90% of CH cases remain unsolved in terms of molecular genetics.

Pathogenesis: Several transcription factors and signaling molecules are involved in the development of the pituitary gland. Mutations in any of these genes may result in CH including *HESX1*, *PROP1*, *POU1F1*, *LHX3*, *LHX4*, *SOX2*, *SOX3*, *OTX2*, *PAX6*, *FGFR1*, *GLI2,* and *FGF8*. Over the last 5 years, several novel genes have been identified in association with CH, but it is likely that many genes remain to be identified, as the majority of patients with CH do not have an identified mutation.

Clinical manifestations: Genotype-phenotype correlations are difficult to establish. There is a high phenotypic variability associated with different genetic mutations. The clinical spectrum includes severe midline developmental disorders, hypopituitarism (in isolation or combined with other congenital abnormalities), and isolated hormone deficiencies.

Diagnosis and treatment: Key investigations include MRI and baseline and dynamic pituitary function tests. However, dynamic tests of GH secretion cannot be performed in the neonatal period, and a diagnosis of GH deficiency may be based on auxology, MRI findings, and low growth factor concentrations. Once a hormone deficit is confirmed, hormone replacement should be started. If onset is acute with hypoglycaemia, cortisol deficiency should be excluded, and if identified this should be rapidly treated, as should TSH deficiency. This review aims to give an overview of CH including management of this complex condition.

Keywords: hypopituitarism, newborn, hypoglycaemia, pituitary gland, hormone deficiencies, septo-optic dysplasia, growth hormone, micropenis

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INTRODUCTION

Congenital hypopituitarism (CH) is defined as the deficiency of one or more hormones produced by the anterior pituitary (AP) or released from the posterior pituitary (PP). Its estimated incidence is between 1 in 4,000 and 1 in 10,000 live births (1).

The pituitary gland is the central regulator of growth, metabolism, reproduction and homeostasis. It is located in the midline of the brain within the sella turcica and consists of three lobes of dual embryologic origin. The adenohypophysis (anterior and intermediate lobes) originates from Rathke's pouch, an invagination of the oral ectoderm, whereas the neurohypophysis (posterior lobe) develops from the neural ectoderm of the ventral diencephalon.

The AP consists of five different cell lineages producing six hormones: somatotrophs (growth hormone, GH), gonadotrophs (follicle stimulating hormone, FSH, and luteinising hormone, LH), corticotrophs (adrenocorticotropic hormone, ACTH), thyrotrophs (thyroid stimulating hormone, TSH), and lactotrophs (prolactin, PRL). The intermediate lobe contains melanotrophs, which secrete proopiomelanocortin (POMC), a major precursor to endorphins, and melanocyte-stimulating hormone (MSH). The PP lobe releases two hormones, oxytocin and antidiuretic hormone (ADH, also known as vasopressin), which are produced in the hypothalamus (supraoptic and paraventricular nuclei) and transported axonally via the pituitary stalk to be stored and released from the PP.

The hypothalamic parvocellular neurosecretory system is responsible for the release of specific AP hormones. It consists of neurons secreting thyrotrophin-releasing hormone (TRH) stimulating secretion of TSH and PRL, corticotrophin-releasing hormone (CRH) that acts to stimulate the secretion of ACTH, gonadotrophin releasing hormone (GnRH) that stimulates release of FSH and LH, growth hormone releasing hormone (GHRH) that stimulates the secretion of GH, somatostatin (SS) that negatively regulates GH secretion, and dopamine that inhibits secretion of PRL. These hypothalamic factors are rapidly transported to the AP via the hypophyseal portal blood system (2, 3).

The aim of this review is to describe the range of mechanisms underlying CH, clinical findings during the neonatal period, diagnosis, treatment, and future therapeutic options.

ETIOLOGY

CH may occur due to developmental defects of the pituitary gland, in some cases as a result of genetic mutations. Acquired forms of hypopituitarism, secondary to perinatal or neonatal events, rarely occur in the neonatal period. CH may present as isolated or combined pituitary hormone deficiencies (CPHD), and may be part of a syndrome involving extra-pituitary abnormalities.

In the majority of cases, the etiology of CH is unknown. The overall incidence of genetic mutations in these patients is low (16% of cases can currently be explained by mutations in known genes) indicating that many genes remain to be identified (4). *PROP1* mutations are the most frequent known cause of both familial and sporadic congenital CPHD. Mutations in other genes have also been described, but appear to be much rarer. However, it is likely during the next few years that novel genetic determinants of pituitary disorders will probably be identified with the availability of next generation sequencing technology (5).

EMBRYOLOGY AND GENETICS

The development of the pituitary gland is a multifactorial process that results from the temporo-spatial interactions of transcription factors and signaling molecules. These occur in distinct and sequential developmental steps. Although direct evidence in humans is lacking, the process of pituitary development is highly conserved across all vertebrate species including rodents, and development of the mouse pituitary, in particular, is well-characterized (6).

Pituitary gland development starts at an equivalent human gestational age of 4–6 weeks and occurs in 4 stages: (i) the pituitary placode, (ii) the rudimentary Rathke's pouch, (iii) the definitive Rathke's pouch, and (iv) the mature pituitary gland.

In the mouse the pituitary placode appears at embryonic (E) day 7.5, located ventrally to the anterior neural ridge and next to the future hypothalamo-infundibular region, which will give rise to the roof of the oral cavity. Initial pituitary development consists of a thickening of the roof of the oral ectoderm at E8.5. By E9.0 it invaginates dorsally and the rudimentary Rathke's pouch is formed (7). The definitive Rathke's pouch, formed by E10.5, gives rise to the anterior and intermediate lobes. The posterior lobe is derived from the posterior part of the developing diencephalon. By E12.5, the precursors of the hormone-secreting cells start to proliferate ventrally from the pouch to constitute the future AP.

Normal pituitary organogenesis requires apposition between the rudimentary Rathke's pouch and the diencephalon. This is critical as the induction and correct formation of the pouch requires at least two sequential inductive signals from the diencephalon (8, 9). Bone Morphogenetic Protein 4 (Bmp4) is the first secreted signaling molecule. Fibroblast Growth Factor 8 (Fgf8) that is the second signal activates two key regulatory genes, LIM homeobox 3 (*Lhx3*) and LIM homeobox 4 (*Lhx4*) that play a critical role in the development of the rudimentary pouch into a definitive pouch (10–12).

These signaling molecules are derived from different embryogenic origins: the ventral diencephalon (Bmp4, Fgf8, Fgf4, Nkx2.1, Wnt5α), the oral ectoderm (Sonic Hedgehog, Shh, the surrounding mesenchyme (Bmp2, Chordin) and the pouch (Bmp2, Wnt4) (13, 14).

Transcription factors expressed early in pituitary organogenesis include *Hesx1*, *Lhx3*, *Lhx4*, *Sox2*, *Sox3*, *Gli2*, and *Otx2*. *Prop1* and *Pou1f1* (previously known as *Pit1*) are implicated in the later stages.

Further cell determination and specification rely on the expression and interaction of multiple signaling molecules and transcription factors (15) and these will be expanded upon in the following section.

CORRELATION BETWEEN PHENOTYPE-GENOTYPE

The variety of phenotypes seen in patients with CPHD is a reflection of the close developmental relationships seen during organogenesis of the pituitary gland, eye, optic nerve, ear, nose, and cranial nerve ganglia.

As a general rule, genetic mutations in genes involved in early development (*HESX1*, *LHX3*, *LHX4*, *SOX2*, *SOX3*, *GLI2*, and *OTX2*) tend to be part of a syndrome that includes extra-pituitary defects and midline abnormalities such as cleft lip and/or palate, as well as CH (**Table 1**). In contrast, mutations in the genes implicated in later stages (*PROP1* and *POU1F1*) result in variable phenotypes of CPHD without any extra-pituitary defects (16) (**Table 2**).

Different gene mutations can result in the same phenotype and different phenotypes can be secondary to the same single genetic mutation. Therefore, the clinical phenotype and associated morphological findings are crucial in the investigation of the underlying genetic mutations of cases of congenital hypopituitarism.

Syndromic Hypopituitarism

Syndromic forms of CH are mainly due to mutations in transcription factors implicated during early pituitary development as listed in **Table 1** and described in detail below.

Septo-Optic Dysplasia and Its Variants

Septo-optic dysplasia (SOD; de Morsier Syndrome) is an extremely heterogeneous and complex disorder defined by the presence of at least 2 of the following: (i) optic nerve hypoplasia (ONH), (ii) midline abnormalities seen in brain and pituitary MRI [mainly agenesis of the corpus callosum (ACC) and absence of the septum pellucidum], (iii) pituitary hypoplasia with hypopituitarism. Its estimated prevalence is 1 in 10,000 live births (17–19).

To date, transcription factors, such as *HESX1*, *SOX2*, *SOX3,* and *OTX2*, are the most common genes implicated in the etiology of SOD. Genetic mutations implicated in Kallmann syndrome (KS), such as *KAL1*, *FGFR1*, *PROKR2,* and *FGF8*, have also been recently identified in patients with SOD (16, 20, 21).

HESX1

The transcription factor *HESX1* is a member of the paired-like class of homeodomain proteins, the initial activation of which may be dependent upon *LHX1* and *OTX2*. *Hesx1* is one of the earliest markers of the pituitary primordium and can be detected in the anterior forebrain from E7.5 to E8.5 and in the Rathke's pouch from E8.5 to E135 (22). From E12, it is rapidly downregulated and becomes undetectable by E13.5 (23).

Hesx1 is a transcriptional repressor and its down-regulation activates other downstream genes such as *Prop1*, suggesting that both function as opposing transcription factors.

Targeted disruption of *Hesx1* in mice results in anophthalmia or microphthalmia and midline neurological defects (such as absent septum pellucidum and pituitary hypoplasia), reminiscent of SOD (24).

The first homozygous missense mutation reported in *HESX1* (p.R160C) was described in two siblings with SOD born to consanguineous parents who presented with ACC, ONH, a hypoplastic AP gland and complete panhypopituitarism (25–27).

Since then, several homozygous and heterozygous *HESX1* mutations have been described. There is no clear genotype-phenotype correlation, and clinical features range from idiopathic GHD to CPHD, associated in some cases with anomalies such as SOD and pituitary malformations (28–33). MRI findings are variable, including a hypoplastic or aplastic AP and an ectopic posterior pituitary (EPP).

The phenotype of those patients with heterozygous mutations in *HESX1* tends to be milder presenting with isolated GHD and an ectopic or undescended posterior PP, although midline forebrain abnormalities can also be seen.

The majority of the cases are sporadic and just around 1% of the patients with SOD present with genetic mutations in *HESX1* (34, 35). In some patients, the penetrance is variable, suggesting the impact of additional genetic, or environmental factors.

SOX2

SOX2 is a transcription factor member of the SRY-related HMG box (SOX) family. It is expressed at 4.5–9 weeks of human pituitary development within Rathke's pouch and is maintained throughout AP development as well as in the diencephalon.

It is expressed throughout the developing central nervous system as well as in sensory placodes, inner ear, cochlea and in the developing lens, retina, and optic nerve (36–44).

SOX2 is extremely important in the maintenance of pituitary progenitor cells and its differentiation into all hormone-producing lineages (45).

The pituitary phenotype associated with murine *Sox2* loss of function mutations usually includes GH, TSH, and gonadotrophin deficiencies (46).

Heterozygous *de novo* mutations in humans have been observed in several patients with hypogonadotropic hypogonadism, bilateral, often severe, anophthalmia/microphthalmia, small corpus callosum, hippocampal abnormalities, and variable mental retardation (47–51). Esophageal atresia has also been reported (52, 53). The pituitary phenotype occasionally includes GH deficiency (GHD).

Imaging of the hypothalamo-pituitary region can show morphological anomalies such as hippocampal abnormalities, hypoplasia of the corpus callosum, hypothalamic hamartoma, and pituitary enlargement that is reminiscent of tumors (54).

SOX3

SOX3 is also a member of the SRY-related HMG box (SOX) family. It is located on the X chromosome (Xp27.1) and is expressed along the full length of the central nervous system including the brain and spinal cord. *SOX3* dosage is critical for normal hypothalamic-pituitary development and both under- and over- dosage of the gene can lead to hypopituitarism (4, 55, 56).

Male patients present with variable hypopituitarism (CPHD or idiopathic GHD) and infundibular hypoplasia, an ectopic/undescended posterior pituitary (PP) and abnormalities

TABLE 1 | Mutations and characteristics of genes involved in syndromic hypopituitarism.

	Transcription factor	Inheritance	Hormone deficiencies	MRI	Phenotype
Septo-optic dysplasia and its variants	*HESX1*	AR, AD	Isolated GHD CHPD	APH EPP ACC	SOD and its variants
	SOX2	AD	LH, FSH deficiency Variable GHD	APH Thin corpus callosum Hippocampal abnormalities Hypothalamic hamartoma Slow progressing hypothalamo-pituitarytumour	Anophthalmia/microphthalmia Esophageal atresia Genital tract abnormalities Sensorineural hearing loss Hypothalamic hamartoma Spastic diplegia Mental retardation Dentalanomalies
	SOX3	X-linked	Pan hypopituitarism GH, TSH, ACTH, LH, and FSH deficiencies Isolated GHD	APH EPP Absent pituitary stalk Persistent craniopharyngeal canal Infundibularhypoplasia	Mental retardation Craniofacial abnormalities Hearing impairment
	OTX2	AD	Isolated GHD CPHD (GH, TSH, PRL, LH, FSH deficiencies)	Normal APH EPP Chiari Syndrome	Eye malformations (bilateral anophthalmia or severe microphthalmia, or coloboma) Developmental delay Seizures
	PAX 6	AR	GHD ACTH deficiency FSH, LH deficiency	APH	Eye malformations
	BMP4	AR	CPHD	Cerebellar abnormalities Partial ACC	Eye malformations Sensorineural hearing loss Developmental delay Spondyloepiphyseal dysplasiatarda
	FGFR1	AD	CPHD (GH, TSH, ACTH, LH, FSH deficiency) with DI	APH EPP Stalk thin or normal ACC	SOD Eye malformations Cleft lip/palate Brachydactyly Central incisor Kallman Syndrome
	ARNT2	AR	DI, ACTH, GH, and TSH deficiencies CPHD	APH Absent PP Stalk thin ACC Frontal and temporal lobe hypoplasia Large Sylvian fissure	Eye malformations Microcephaly Renal abnormalities Seizures
Holoprosencephaly	*GLI2*	AD Haplo-insufficiency	CPHD GH, TSH, ACTH, LH, and FSH deficiencies Isolated GHD	Holoprosencephaly APH EPP or normal PP Hypoplastic corpus callosum Cavum septumpellucidum	Midfacial defects Cleft lip/palate Single central incisor Postaxial polydactyly ONH
	FGF8	AD AR	Hypopituitarism LH, FSH deficiencies TSH deficiency ACTH deficiency DI Borderline peak GH concentration	Holoprosencephaly ACC ONH	Kallman Syndrome (HH + Anosmia) Moebius syndrome Spastic diplegia Developmental delay SOD
PSIS	*OTX2*	AD	IGHD CPHD (GH, TSH, PRL, LH, FSH deficiencies)	Normal APH EPP Chiari syndrome	Anophthalmia (bilateral/unilateral) Coloboma Retinal dystrophy Normal eye phenotype Bilateral severe microphthalmia Seizures Developmental delay

(Continued)

TABLE 1 | Continued

	Transcription factor	Inheritance	Hormone deficiencies	MRI	Phenotype
	PROKR2	AD AR	GH, TSH, ACTH, LH, FSH deficiencies GHD	PSIS APH or normal AP EPP Absent stalk Dysgenesis of corpus callosum Other: Schizencephaly Cerebellar hypoplasia Hypoplastic optic discs and nerves	Neonatal hypoglycaemia Micropenis Facial asymmetry SOD Kallman syndrome
	GPR161	AR	GHD	PSIS APH EPP Other: Emptysella	Alopecia Short 5th finger Ptosis left eye
Other Syndromes	*IGSF1*	X linked	TSH, GH, PRL deficiencies	/	Macroorchidism Delayedadrenarche
	NFKB2	AD	ACTH, TSH, GH deficiencies	/	Variable Immune deficiency (DAVID Syndrome)
	PITX2	AD	LH, FSH deficiencies GH insufficiency	APH Hypoplasia sellaturcica	Axenfeld—Rieger Syndrome: Anterior eye chamber Dental hypoplasia Craniofacial dysmorphism Protuberantumbilicus
	CHD7	AD	GH, TSH, FSH, LH deficiencies	EPP	CHARGE Syndrome
	LHX3	AR	CPHD (GH, TSH, FSH, LH, PRL, deficiencies variable ACTH deficiency)	Pituitary hypo- or hyperplasia Normal PP and stalk	Skeletal abnormalities Abnormal head and neck rotation (70%) Vertebral abnormalities—short cervical spine (50%) Sensorineural deafness (mild tosevere)
	LHX4	AD	Isolated GHD CPHD (GH, TSH, ACTH deficiencies, variable FSH, LH deficiencies)	APH PP normal or EPP Small sella turcica Corpus callosum hypoplasia Cerebellar abnormalities Chiari Syndrome	Lung defects—respiratory distress

ACC, Agenesis corpus callosum; ACTH, Adrenocorticotropic hormone; AD, Autosomal Dominant; APH, Anterior Pituitary Hypoplasia; AR, Autosomal Recessive; CPHD, Combined Pituitary Hormone Deficiencies; EPP, Ectopic Posterior Pituitary; FSH, Follicle-stimulating hormone; GH, Growth Hormone; GHD, Growth Hormone deficiency; LH, Luteinizing Hormone; ONH, Optic Nerve Hypoplasia; PP, Posterior Pituitary; PRL, prolactin; PSIS, pituitary stalk interruption syndrome; SOD, Septo-optic Dysplasia; TSH, thyroid stimulatinghormone.

of the corpus callosum. Intellectual disability is also frequently reported in these patients (4, 57, 58). Patients with duplication of *SOX3* can present with GHD without other pituitary deficiencies (59). Loss of function polyalanine expansions and gene deletions are associated with hypopituitarism including GH, TSH, ACTH, and gonadotrophin deficiencies. In terms of neuroradiological features, AP hypoplasia, an absent pituitary stalk, and ectopic EPP are other findings associated with *SOX3* sequence variants or whole gene deletions/duplications. Persistence of the craniopharyngeal canal has been reported in association with a *SOX3* deletion (60).

OTX2

Orthodenticle homeobox 2 (*Otx2*) is a transcription factor gene involved in brain, eye, nose and ear development (61, 62). It is expressed from E10.5 to E14.5 in the ventral diencephalon, from E10.5 to E12.5 in Rathke's pouch, and then becomes undetectable at both sites from E16.5 (63).

OTX2 regulates various transcription factors implicated in brain, eye and pituitary development, including *RX1*, *PAX6*, *SIX3*, *LHX2*, *MITF*, *GBX2*, and *HESX1* in order to coordinate cell determination and differentiation.

As *OTX2* is essential in retinal development, many patients with *OTX2* mutations and pituitary hormone deficiencies also present with a variety of ocular abnormalities. In humans, heterozygous *OTX2* mutations or gene deletions have been implicated in the etiology of 2–3% of anophthalmia/microphthalmia syndromes (64–67).

There is no clear genotype-phenotype correlation, even among patients with the same mutation. The pituitary phenotype ranges from partial to complete GHD and brain MRI can show a normal or hypoplastic AP, normal or EPP, and Chiari malformation (68, 69). In those patients without ocular involvement, who have CPHD and a small AP with or without an undescended PP, mutations in *OTX2* have been rarely reported (70). One of these rare cases is the

TABLE 2 | Mutations and characteristics of genes involved in non-syndromic Hypopituitarism.

Transcription factor	Inheritance	Hormone deficiencies	MRI	Phenotype
PIT1/POU1F1	AD, AR	GH, PRL, and TSH deficiencies	APH or normal AP Normal PP Normal infundibulum No extra pituitary abnormalities	No extra- pituitary abnormalities TSH deficiency may present early or develop muchlater
PROP 1	AR	GH, TSH, PRL, LH, FSH deficiencies Evolving ACTH deficiency	APH, normal or enlarged AP (transient, may change over time) Normal PP Normals talk	No extra-pituitary abnormalities Variable time of onset and severity of pituitary deficiencies

ACC, Agenesis corpus callosum; ACTH, Adrenocorticotropic hormone; AD, Autosomal Dominant; APH, Anterior Pituitary Hypoplasia; AR, Autosomal Recessive; CPHD, Combined Pituitary Hormone Deficiencies; EPP, Ectopic Posterior Pituitary; FSH, Follicle-stimulating hormone; GH, Growth Hormone; GHD, Growth Hormone deficiency; LH, Luteinizing Hormone; ONH, Optic Nerve Hypoplasia; PP, Posterior Pituitary; PRL, prolactin; PSIS, pituitary stalk interruption syndrome; SOD, Septo-optic Dysplasia; TSH, thyroid stimulatinghormone.

p.N233S mutation where patients may not exhibit an ocular phenotype (71).

PAX6

PAX6 is an early dorsal marker of early AP gland and its expression is required for somatotrope, lactotrope, and thyrotrope development. It is also an important regulator of eye development, and heterozygous mutations in humans cause congenital eye anomalies (72). Recently, *PAX6* mutations have been reported to be associated with impaired pituitary function (ACTH deficiency, hypogonadotropic hypogonadism, and GHD) (73–75).

BMP4

Bone morphogenetic protein 4 (Bmp4) is the first secreted molecule detected in the prospective infundibulum at E8.5. It is essential for Rathke's pouch formation and maintenance. It is expressed in the optic vehicle, in the diencephalic floor and in the medial ganglionic eminence and in developing limbs. A recent study that included patients with eye abnormalities identified *BMP4* mutations in a familial case of anophthalmia, retinal dystrophy, brain malformation, and poly/syndactyly (76). Deletions in *BMP4* were associated with bilateral anophthalmia/microphthalmia, in association with hypothyroidism, deafness, developmental delay, and cerebellar and pituitary abnormalities.

FGFR1

FGF receptor 1 (FGFR1), a tyrosine kinase receptor for FGF, is the most important receptor involved in FGF8 signaling. Mutations in *FGFR1* have previously been reported in patients with Kallmann syndrome; more recently, *FGFR1* variants have been associated with CPHD, absent corpus callosum, SOD, and midline defects (77, 78).

ARNT2

Aryl hydrocarbon receptor nuclear translocator 2 (ARNT2) belongs to the HLH-PAS (Per-ARNT-Sim homology) subfamily of transcription factors. *Arnt2* is find on the hypothalamus, eye (neural retina), and kidney and urinary tract in rodents, and this expression pattern recapitulates that observed in humans (76).

Holoprosencephaly: Gli2 and FGF8

GLI2, a mediator of SHH signaling, is expressed in the ventral diencephalon inducing BMP4 and FGF8 expression, and also in the oral ectoderm, inducing pituitary progenitors. *GLI2* mutations are associated with holoprosencephaly (HPE) or HPE-like features with craniofacial anomalies, pituitary abnormalities and polydactyly (79–81).

Fibroblast growth factor 8 (*Fgf8*) is a member of the FGF family of signaling molecules that are involved in pituitary organogenesis. It is expressed in the infundibulum at E9.5, 1 day after the expression of Bmp4 (20, 82) and is important in midbrain development.

FGF8 mutations are associated with Kallmann syndrome and have more recently been described in association with recessive HPE, craniofacial defects, and hypothalamo-pituitary dysfunction (83).

Hypopituitarism With Spine Abnormalities: LHX3

Expression of the LIM homeobox 3 (*LhX3*) gene, a member of the LIM class of homeodomain proteins, is detected early during AP development at E9.5 (Ratkhe's pouch, ventral hindbrain, and spinal cord) and persists in the mature pituitary gland. It is one of the earliest markers implicated in the anterior and intermediate lobes development and its expression plays an important role for the formation of gonadotrophs, thyrotrophs, somatotrophs, and lactotrophs (84).

Mice with homozygous mutations of *Lhx3* die soon after birth as a result of pituitary aplasia whereas those with heterozygous mutations have no abnormalities (85).

In humans, 14 homozygous (86–94) or compound heterozygous *LHX3* mutations (10) and a heterozygous variant (95) have been reported to date.

Commonly, patients with *LHX3* mutations present with GH, TSH, and FSH/LH deficiencies while ACTH deficiency is reported in 50% of cases (94).

The phenotype varies depending on which part of the gene is affected. If the mutation affects the entire gene or protein, the LIM domains or the homeodomain, patients will present with syndromes involving the nervous and skeletal systems, whereas if the mutation affects the carboxyl terminus of LHX3 protein

alone, only pituitary dysfunction will be present. LHX3 is also required for inner ear development.

Extra-pituitary phenotypes can include a short neck with abnormal head and neck rotation (70% of cases), vertebral abnormalities (50% of cases) including a rigid cervical spine, flattened lumbar vertebrae, thoracic kyphosis, and progressive scoliosis, and variable degrees of sensorineural hearing loss (50% of cases). Developmental delay or learning difficulties have also been reported in nearly 40% of the patients. Two of the reported patients also had respiratory distress. Heterozygous family members are largely unaffected, although a recent publication has described a mild limitation of neck movement in a heterozygous carrier (10).

MRI findings can vary from a normal MRI (10% of the cases) to aplasia or hypoplasia of the AP, a hypointensity suggestive of microadenoma, and enlargement with a hyperintense signal (91).

Hypopituitarism With Cerebellar Abnormalities: LHX4

Lhx4 is closely related to Lhx3 and is expressed in the developing brain and spinal cord (96). It is also detected during early stages (E9.5 in Rathke's pouch and E12.5 in the anterior part of the pituitary) and is subsequently found in the future anterior lobe, with a decrease in expression by E15.5.

The AP gland in patients with *LHX4* mutations is hypoplastic, containing all the differentiated cell types but in reduced numbers. Other brain abnormalities can also be present such as an EPP and a hypoplastic sella turcica as well as corpus callosum hypoplasia or Chiari syndrome (97–105).

In humans, the phenotype can range from isolated GHD to complete panhypopituitarism (106). Several sporadic or familial *LHX4* mutations have been reported to date. Of note, four patients also presented with respiratory distress (76, 103, 107) and one presented with a cardiac defect (76). Several of the variants are variably penetrant, although the underlying mechanism remains to be established.

A lethal neonatal phenotype (severe panhypopituitarism associated with anterior pituitary aplasia and EPP, mild facial hypoplasia, undescended testes, and severe respiratory distress) has been recently described, secondary to a homozygous mutation (107).

Pituitary Stalk Interruption Syndrome (PSIS)

PSIS is a congenital defect of the pituitary gland characterized by the triad of (i) a thin pituitary stalk, (ii) an EPP gland, and (iii) hypoplasia or aplasia of the AP gland identified by MRI. Patients with PSIS may present with either isolated GHD or CPDH (22). Genetic alterations in *HESX1*, *LHX4*, *OTX2*, *SOX*3, and *PROKR2* have been reported in patients with PSIS, amongst others.

PROKR2, a G protein-coupled receptor essential for proper neuronal migration and angiogenesis, is involved in sex development and olfactory bulb development in mice. In humans, patients with mutations in *PROKR2* can present with hypogonadotropic hypogonadism or Kallmann syndrome. More recently, mutations have been associated with variably penetrant hypopituitarism including SOD (108–112). As described previously (section Septo-optic dysplasia and its variants), *OTX2* gene defects were also reported in patients with no ocular abnormalities (71).

GPR161, an orphan member of the G protein–coupled receptor family, has also been recently identified in patients with PSIS. GPR161 is widely expressed in both mouse and human during the early stages of embryogenesis including the neural folds, the pituitary and the hypothalamus.

It is a key negative regulator of the SHH pathway, the pituitary target of which is GLI2. It has been suggested that gain-of-function mutations of *GPR161* could lead to abnormal pituitary development by repressing the SHH pathway (22).

A homozygous missense mutation p.L19Q in *GPR161* has been recently described in two female siblings with short stature due to GHD associated with AP hypoplasia and an empty sella with an EPP. They also had a short 5th finger, congenital alopecia, and ptosis of the left eye (113).

Central Hypothyroidism and Macroorchidism

IGSF1 is located at Xq26 and is expressed in Rathke's pouch, in the pituitary gland (present in GH, prolactin and TSH-secreting cells), and testis.

IGSF1 mutations (loss of function or deletions) cause an X-linked syndrome. Male patients with mutations in *IGSF1* present with a characteristic phenotype that consists of congenital central hypothyroidism, delayed puberty, and adult macroorchidism. PRL and/or GH deficiencies have also been reported in some cases (114, 115). Some female patients with heterozygous mutations in *IGFS1* present with central congenital hypothyrodism, PRL deficiency, and delayed puberty.

Deficient Anterior Pituitary Function With Variable Immune Deficiency (DAVID) Syndrome

NFKB2 belongs to the NF-κB family, which consists of a collection of evolutionarily conserved transcription factors involved primarily in development including the anterior pituitary gland, immunity, and oncogenesis.

Patients with *NFKB2* mutation present with deficit in AP gland function and common variable immune deficiency, a novel disorder called DAVID syndrome (116). However, the precise mechanism underlying endocrine deficits remains largely unclear.

Axenfeld—Rieger Syndrome

Pitx2, a homeobox gene, is detected in the stomodeum at E8, Rathke's pouch at E10.5 and 2 days after in the anterior and intermediate lobes. Patients affected with *PITX2* mutations present with Axenfeld–Rieger Syndrome which is characterized by eye, craniofacial, dental, cardiac, and umbilical anomalies (117).

CHARGE Syndrome and Pituitary Deficiencies

CHARGE syndrome is rare autosomal dominant disorder that affects multiple organs. It is characterized by ocular coloboma, cardiac defects, choanal atresia, growth, and developmental delay and ear abnormalities. EPP and hypopituitarism in patients with CHARGE syndrome has been recently reported in association with two novel *CHD7* variants (118).

Isolated ACTH Deficiency

Congenital isolated ACTH deficiency is mainly due to recessive mutations in *TBX19* (formerly *TPIT*) which are responsible of approximately 65% of the cases of isolated ACTH deficiency diagnosed during the first month of life (119). Neonates present with severe hypoglycaemia that can result in seizures and prolonged cholestatic jaundice. Biochemically this is characterized by low basal ACTH and cortisol concentrations and a poor ACTH response to corticotropin releasing hormone (CRH). It is extremely important to diagnose as this can be a potential cause of death during the neonatal period if no replacement treatment is started (120).

Non—syndromic Combined Pituitary Hormone Deficiencies

Mutations in *PROP1* and *POU1F1* constitute the main genetic cause found in patients with non-syndromic GHD or CPHD (**Table 2**).

Mutations in PROP1

Prop1 (prophet of PIT-1), a member of the paired-like family of homeodomain transcription factors, is the earliest expressed pituitary-specific transcription factor. It is detected within Rathke's pouch by E10, peaks at E12 and it disappears by E15.5 (121).

Prop1 can act as both a transcriptional repressor (for *Hesx1* expression) or a transcriptional activator for *Pou1f1* (121, 122). Mice with *Prop1* over expression present with delayed puberty as a consequence of a delay in the differentiation of gonadotrophs (123).

The most frequent genetic cause of CPHD are recessive mutations in *PROP1* (124–130). The most common of these is a 2 base pair deletion within exon 2, which results in a frameshift at codon 101 and introduction of a termination codon at position 109 (130).

Recessive *PROP1* mutations are associated with GH, TSH, PRL and gonadotrophin deficiencies which vary in onset and severity. ACTH deficiency usually occurs later. They vary in the time of onset and severity and therefore it is important to have ongoing clinical surveillance.

GHD and growth delay is usually present during the first years of life in patients with *PROP1* mutations, however, there is a case report of a patient who had normal growth and achieved a normal adult height without GH treatment (131).

Both TSH and gonadotropin deficiency can appear at birth or later in life (132–134). Some patients may present with micropenis and undescended testes at birth and some others with delayed puberty. Spontaneous puberty can also be seen (135–137).

The majority of the patients do not have ACTH or cortisol deficiency during the first years of life but this can evolve later and ongoing surveillance is therefore needed (135, 138).

MRI shows variable pituitary morphology. The commonest finding is a normal pituitary stalk and posterior lobe with a small or normal AP gland. An enlarged AP gland has also been described with posterior regression (137).

TABLE 3 | Clinical presentation of hypopituitarism in a neonate.

Symptom/sign	Pituitary hormone deficiency
Poor feeding	GH, ACTH
Poor weight gain	GH, ACTH, DI
Jitteriness	GH, ACTH
Lethargy	GH, ACTH
Seizures	ACTH
Recurrent sepsis	ACTH
Apnoea	ACTH
Conjugated jaundice	ACTH
Prolonged unconjugated jaundice	TSH
Temperature instability	TSH
Respiratory difficulties	TSH
Polyuria	DI
Polydipsia	DI
Undescended testes	Gonadotropin
Micropenis	Gonadotropin, GH

Mutations in POU1F1

POU1F1, a member of the POU family, is expressed later during the pituitary organogenesis (E14.5) and persists during adulthood (139). It plays a crucial role in the regulation of the genes encoding GH, PRL, and TSH-beta and the time of onset and severity also varies. Gonadotroph and corticotroph axes usually remain functional.

Patients tend to present first with GH and prolactin deficiencies during the first years of life whereas TSH deficiency tends to present later (140).

POU1F1 mutations are mainly recessive. However, dominant mutations have been recently described being the most frequent p.R271W mutation (141).

MRI reveals a normal or small AP gland; The PP and infundibulum are normal and no midline abnormalities have been reported (140, 142).

CLINICAL PRESENTATION, DIAGNOSIS, AND TREATMENT

Given the crucial regulatory role of the pituitary gland, prompt recognition of those neonates at risk of CH is important, as a delay in replacement therapy can have devastating consequences. Identifying neonates with CH can be challenging because they often present with non-specific symptoms such as hypoglycaemia, prolonged jaundiced, poor weight gain, temperature dysregulation, electrolyte abnormalities, and haemodynamic instability (**Table 3**).

Birth weight and length tend to be normal, although GHD can lead to a slight reduction in birth weight. The clinical presentation and its severity depend on the number of hormones affected. These patients can have associated genital abnormalities, eye malformations, and/or midline defects.

Neonates with ACTH deficiency can present with cholestasis during the first 2 weeks of life. To understand the association

between cholestasis and ACTH deficiency it is important to remind that cortisol increases bile flow and therefore, its deficiency will cause abnormalities in the synthesis and transport of bile acid leading to cholestasis in some cases.

A rise in transaminase concentrations can be seen after 2–4 weeks but GGT remains normal.

Once cortisol replacement treatment is started, cholestasis tends to resolve in around 10 weeks' time. In those cases where a liver biopsy is performed due to a delay in the diagnosis of CH, this shows canalicular cholestasis, and histopathology reveals mild portal eosinophilic infiltration.

Investigations to diagnose CH include baseline pituitary function tests (+ dynamic tests if indicated) and brain MRI. Genetic testing should also be considered.

However, the sensitivity and specificity of laboratory tests are limited in the newborn, especially in premature infants due to hypothalamo-pituitary axis immaturity, and lack of normative values. Additionally, GH stimulation tests are contraindicated under the age of 1 year.

A high index of suspicion for CH and early treatment in these patients is vital to avoid clinical decompensation. Treatment involves the physiological replacement of the relevant hormone deficiencies and requires close lifelong monitoring.

Individual hormone deficiencies are discussed in detail in the following section.

ACTH Deficiency

Clinical Presentation

Neonates can present with failure to thrive, severe hypoglycaemia and cholestasis.

Diagnosis

As neonates do not have a circadian rhythm [reported to be established at around 2 months of age (143), or after 6 months of age (142), morning cortisol concentrations are not useful in evaluating ACTH deficiency in this population. Although cortisol deficiency related hypoglycaemia is severe, low cortisol concentrations at the time of hypoglycaemia have low specificity for the diagnosis of adrenal insufficiency and therefore should not be the only test for the diagnosis of ACTH deficiency (144). Many patients require a dynamic assessment (ACTH stimulation test using tetracosactide hexaacetate). The dose of tetracosactide hexaacetate used to diagnose central adrenal insufficiency, the timing for collection of blood samples for cortisol measurement, and the cut-off peak cortisol concentration for both the low-dose and standard ACTH test are the subject of much controversy. Stimulated cortisol concentrations $\geq$18 mg/dL (497 nmol/L) are indicative of a normal hypothalamo-pituitary-adrenal axis (145).

Treatment

In preterm infants, daily cortisol production is known to be $\sim$7 mg/m^2/day on the fifth day and $\sim$6 mg/m^2/day in the second week. When a neonate is diagnosed with ACTH deficiency, treatment needs to be started immediately. The treatment of choice is hydrocortisone due to its less potent side effects in terms of growth and bone health compared to other glucocorticoids. The starting dose of hydrocortisone is 9–12 mg/m^2/day in 3–4 divided doses. This dose is higher compared to older infants because neonates have greater cortisol secretion rates. The dose can then be titrated with age. Prior to discharge, education for families about sick day rules and emergency dosing is important. In event of illness or stress, hydrocortisone doses should be doubled or even tripled. In event of an emergency, poor tolerance of oral hydrocortisone, or a suspected adrenal crisis, intramuscular hydrocortisone must be administered. The dose is age-dependent (<1 year 25 mg, 1–5 years 25–50 mg, >5 years 100 mg) and oral glucose should also be given to correct any associated hypoglycaemia. Those patients that cannot tolerate oral hydrocortisone require admission for intravenous hydrocortisone (1–2 mg/kg every 4–6 h). One they are able to tolerate oral hydrocortisone this is commenced at triple or double maintenance dose, and gradually weaned to maintenance depending on clinical improvement.

It is also important to highlight that cortisol deficiency can mask DI as cortisol is needed for water excretion. DI may develop after starting treatment with hydrocortisone and therefore close monitoring of fluid balance and electrolytes is important after starting glucocorticoid therapy (145).

Novel treatments such as continuous subcutaneous hydrocortisone infusion therapy, which may be difficult in neonates due to limited subcutaneous fat for insertion of the cannula, and sustained release hydrocortisone preparations aimed at mimicking physiological cortisol secretion remain to be established as potential therapies (146).

TSH Deficiency

Etiology

Defects in TRH or TSH signaling are responsible of isolated central congenital hypothyroidism. As mentioned before, the most frequent genetic cause of isolated central congenital hypothyroidism is *IGSF1* gene mutation (147). Less common causes include genetic defects in TSH production, that is, mutations in the TRH receptor or TSH-B subunit (112, 148). More recently, mutations in *TBL1X* have been described in association with TSH deficiency.

Clinical Presentation

Newborns with TSH deficiency can present with prolonged physiological jaundice and low energy levels/sleepiness. Other findings such as temperature dysregulation, umbilical hernia, dry skin, bradycardia, macroglossia, and constipation may also be present.

X-linked central hypothyroidism due to *IGSF1* mutation is also later associated with delayed puberty and adult macroorchidism (149).

Diagnosis

Thyroid hormone is critical for normal brain development within the first 3 years of life, and therefore a prompt diagnosis is essential so that treatment can be commenced rapidly. Central hypothyroidism is characterized by the biochemical picture of low free T4 and usually low TSH (although it can also be inappropriately normal or even slightly elevated).

Treatment

Levothyroxine (LT4) is the treatment of choice in newborns with TSH deficiency at a starting dose between 10 and 15 μg/kg/day (150). However, higher doses will be needed in newborns with cholestasis due to malabsorption. Iron, soy, calcium, and anticonvulsants can also affect LT4 absorption and thus should not be co-administered with them. LT4 should ideally be given on an empty stomach but this is not always practical in neonates and so may need to be given with a small amount of milk. LT4 solution or crushed tablets can be given with water, breastmilk or formula.

For those babies unable to tolerate enteral preparations, intravenous tri-iodothyronine (T3) is available. The recommended intravenous dose is 75% of the total oral LT4 dose (151).

Before starting treatment with LT4, it is extremely important to exclude cortisol deficiency. LT4 increases basal metabolic rate, enhancing cortisol clearance with the subsequent risk of precipitating an adrenal crisis.

Monitoring

T4 concentrations should be monitored every 2–4 weeks during initial period of dose tritration. Thereafter monitoring may reduce in frequency. The aim is to keep fT4 in the mid-upper half of the normal range (152). TSH is not useful for monitoring in these cases.

Gonadotrophin Deficiency

Clinical Presentation

Males present with micropenis, with or without undescended testes. Micropenis refers to a stretched penile length of −2.5 SD from the mean value: <1.5 cm at gestational age 30 weeks, 2 cm at 34 weeks, and <2.5 cm in term babies. Development of female genitalia is not affected by hypogonadotropic hypogonadism (HH) as it is independent of hormone secretion.

Diagnosis

Males

Mini puberty (raised LH and FSH) is seen between 15 days and 6 months old. Testosterone concentrations increase with a peak in the 4–10th week and start to decrease around the 6th month. LH concentrations <0.8 IU/L and testosterone <30 ng/mL between day 5 and 6 months of life are suggestive of the diagnosis. When an hCG test is done to assess testosterone production, penile growth and testicular descent may ensue and need to be documented. There are scant normative data pertaining to hCG tests in the first years of life. However, a study performed in adolescent males suggested that a peak LH concentration < 2.8 IU/L after GnRH stimulation, with a testosterone peak of < 3.6 nmol/L after 3 days of hCG injections and < 9.5 nmol/L after 3 weeks of hCG injections are highly suggestive of hypogonadotrophic hypogonadism (153).

Females

Mini puberty is seen between 15 days and 2 years of age. FSH concentrations < 0.1 IU/L between 15 days and 2 years of life are diagnostic of probable hypogonadotrophic hypogonadism.

Treatment

In newborn male infants, the aim of the treatment is to ensure normal testicular descent, improve penile length and maximize fertility potential. Early treatment is recommended, ideally between 1 and 6 months of age. Testosterone can be given via intramuscular injections or topical gel (153–156). Testosterone injections (cypionate or enanthate) are commenced at a recommended dose of 25 mg every 4 weeks for 3 months. This is followed by clinical evaluation of the stretched penile length. Topical gel containing 5-α Dihydrotestosterone (DHT) is also useful and the recommended starting dose is 1 application (10 mg) every day for 3 months (153). The carer who is applying the testosterone gel should wash hands soon after the administration with soap and water and if the career is a female, the use of gloves is recommended. Cryptorchidism increases the risk of testicular neoplasia and also reduces fertility potential, therefore surgical correction (orchidopexy) is recommended during the first 2 years of life, ideally by 18 months of age (153, 157, 158). Treatment with LH and FSH during the neonatal period still remains under investigation (158–160).

GH Deficiency

Congenital isolated GH deficiency (GHD) has an incidence of 1 in 4,000–1 in 10,000 live births (33), and is the most commonly affected pituitary hormone in childhood.

Etiology

Most of the cases are sporadic but there are four genetic forms that account for 5–30% of cases (161, 162). Congenital isolated GHD can be secondary to genetic mutations in the genes encoding growth hormone (*GH1*) or the growth hormone releasing hormone receptor (*GHRHR*), or in the genes encoding transcription factors SOX3, HESX1, GLI2, OTX2, LHX3, LHX4, PROP1, and POU1F1 (4, 163). Mutations in *GH1* and *GHRHR* may also lead to severe early growth failure with hypoglycaemia. Biallelic mutations in *RNPC3* have also been recently described patients with severe IGHD and AP hypoplasia (23, 164).

Clinical Presentation

Key features of (GHD) include hypoglycaemia and micropenis. It is important to note that GHD does not significantly affect fetal growth, and therefore, affected newborns are usually of normal weight and length at birth, with subsequent post-natal growth failure.

Diagnosis

GH evaluation in a neonate differs from that in an older child. During the neonatal period GH concentrations are higher in the term neonate during the first week of life than throughout childhood but a rapid decrease is seen during the following weeks (165). In contrast, IGF-1 concentrations (stimulated by GH) cannot be used as a screening test in neonates as they remain low for at least the first 15–18 months of age (166). A random GH concentration of less than or equal to 5 ng/mL (5 mcg/L) during the first 7 days of life accompanied by other pituitary hormone deficiencies and/or the classical imaging triad (EPP with AP hypoplasia and an abnormal stalk) is sufficient to

diagnose GHD (165). Binder et al. (167) suggested that a GH cut-off of 7 μg/L as measured on a neonatal screening card by a highly sensitive polyclonal ELISA gave 100% sensitivity and 98% specificity. GH stimulation tests are considered dangerous and are contraindicated during the neonatal period, and a low GH concentration at the time of hypoglycaemia in isolation is not enough to diagnose GHD.

Treatment

In the event of persisting hypoglycaemia, GH treatment can be commenced during the neonatal period with daily subcutaneous recombinant human GH (rhGH) injections in the evening to mimic physiological growth hormone release. The initial recommended dose is between 0.16 and 0.24 mg/kg per week (22–35 mcg/kg per day) (165). Lower doses (10–20 mcg/kg/day) can also lead to excellent responses at this age. GH treatment can contribute to hypoglycaemia recovery and may improve cholestasis during the neonatal period (168).

Monitoring

Subsequent dosing should be individualized by monitoring IGF-I concentrations (at least every 3 months at the beginning). Patients also should be monitored for hypothyroidism and adrenal insufficiency as GH treatment increases metabolism of thyroid hormone and cortisol and may unmask these conditions.

PRL Deficiency

Etiology

Prolactin deficiency is usually due to *POU1F1*, *LHX3*, *OTX2*, and *IGSF-1* gene mutations. It is important to note that some medications can affect PRL concentrations such as dopamine, calcium channel blockers and ranitidine.

Clinical Presentation

Puerperal alactogenesis is the only specific physical finding.

Diagnosis

A random prolactin concentrations <31 ng/mL during the neonatal period supports a diagnosis of PRL deficiency, however, breast tissue should not be palpated prior to a blood sample being taken as the levels could be falsely elevated. Prolactin concentrations are often elevated in association with midline defects.

Treatment

There is no commercially available treatment for PRL deficiency.

Diabetes Insipidus (DI)

Etiology

In most cases of neonatal DI, anatomical defects or autosomal dominant or recessive genetic causes are present. DI is also observed in cases with SOD, corpus callosum agenesis and HPE. Renal concentrating mechanism can also be affected by other factors such as neonatal diabetes, hypercalcaemia, hypokalaemia. It is also important to note that mannitol, dextrose, saline fluids, and imaging contrast mediums can produce osmotic diuresis and secondary polyuria.

Clinical Presentation

The clinical features include polyhydramnios, polyuria, weight loss, irritability, dehydration, and hypernatremia.

Diagnosis

Diagnosis during the neonatal period is challenging as the capacity to concentrate urine is not as efficient as in older children and a water deprivation test is not recommended. Polyuria in DI during the neonatal period is defined as >5 mls/kg/h. Urine osmolality <300 mOsm/kg with a paired serum osmolality >300 mOsm/kg is suggestive of the diagnosis. If the urine osmolality is >600 mOsm/kg, DI is unlikely. The vasopressin test is useful to distinguish between central (CDI) and nephrogenic forms of DI but this can be hazardous during the neonatal period.

Treatment

Fluid therapy alone, without DDAVP, is the recommended management during the neonatal period as it can maintain euvolaemia. However, when CDI is extremely severe, a neonate may not respond to fluid therapy alone and DDAVP might be needed. In some cases of severe CDI, a thiazide diuretic may also be used. DDAVP can result in rapid fluid retention, hyponatremia and secondary cerebral oedema or even death in this vulnerable cohort of patients. Over-treatment is more dangerous than under-treatment and this is why a low starting dose of DDAVP (e.g., 1 μg) is recommended. Close monitoring (electrolytes, paired plasma and urine osmolalities, weight, and clinical examination for signs of fluid retention) is crucial, and dose adjustment will depend on the response to treatment (169). It is important to ensure breakthrough urine output prior to the next dose of DDAVP, in order to avoid severe fluid retention and hyponatraemia. It must also be highlighted that DDAVP may need to be withheld in neonates with concomitant ACTH deficiency who are unable to tolerate or absorb hydrocortisone when unwell (e.g., if vomiting), until appropriate steroid cover is provided. This is because cortisol is required for free water excretion and ongoing therapy with DDAVP without appropriate steroid replacement puts the neonate at risk of water intoxication.

IMAGING: BRAIN AND PITUITARY MRI

MRI of the brain and pituitary gland is recommended in all patients with suspected or confirmed CH. Abnormal brain and pituitary MRI findings do correlate with the severity and evolution of the disease (170).

The pituitary gland in newborns tends to be convex showing high signal intensity on T1-weighted images. As discussed previously, patients with CH usually have abnormal MRI findings ranging from a small AP gland to severe hypoplastic pituitary gland with EPP or undescended PP and an interrupted or hypoplastic pituitary stalk. A "bright spot" identifies the PP gland, however it can be absent in 10% of healthy individuals (170).

Pituitary/brain MRI studies should include qualitative description and dimensions of the AP; location and size of the PP gland, description of the pituitary stalk and comments about extra pituitary structures such as the optic chiasm, septum

pellucidum, and corpus callosum. In order to have a proper description, the best technique is 2-mm to 3-mm thick, high-resolution T1-weighted, and T2-weighted images in coronal and sagittal planes.

The majority of newborns with severe CH show an EPP, abnormal pituitary stalk, and/or AP hypoplasia on MRI. This triad is known as "Pituitary Stalk Interruption Syndrome" (PSIS). Patients with IGHD and PSIS need to be closely monitored for evolving endocrinopathies as they can progress to CPHD.

Other midline brain abnormalities (absent/hypoplastic corpus callosum, absent septum pellucidum, schizencephaly, heterotopia) and ONH may be associated (33, 171).

CONCLUSION

CH can be a life-threatening condition. A high index of suspicion is required for its early identification and treatment. However, early diagnosis during the neonatal period is challenging due to the variable and non-specific presenting symptoms. Red flag symptoms of CH include hypoglycaemia at birth and a micropenis.

In neonates with confirmed or suspected CH, a brain MRI with pituitary views is essential to exclude structural abnormalities. Ophthalmological review is also recommended to evaluate the optic nerves as many cases can have associated ocular abnormalities.

CH is an evolving and lifelong condition and therefore neonates with CH will require long term follow-up in order to detect early evolving endocrinopathies and optimize treatment. For those cases with a positive genetic finding, counseling is recommended (172). Currently, genetic analysis is successful in identifying an aetiological basis only in around 20% of cases (173). However, rapid advances in next-generation sequencing technology will help and improve our understanding of the complex mechanisms involved in congenital hypopituitarism (174). This technological progress is likely to have a positive impact on the clinical care of patients in the future (175).

AUTHOR CONTRIBUTIONS

Edited by LB. Reviewed by HK and MD. All authors contributed to the article and approved the submitted version.

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Endocrine Diseases of Newborn: Epidemiology, Pathogenesis, Therapeutic Options, and Outcome "Current Insights Into Disorders of Calcium and Phosphate in the Newborn"

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The physiology and regulation of bone minerals in the fetus and the newborn is significantly different from children and adults. The bone minerals calcium, phosphate and magnesium are all maintained at higher concentrations *in utero* to achieve adequate bone accretion. This is an integral component of normal fetal development which facilitates safe neonatal transition to post-natal life. When deciphering the cause of bone mineral disorders in newborns, the potential differential diagnosis list is broad and complex, including several extremely rare conditions. Also, significant discoveries including new embryological molecular genetic transcription factors, the role of active placental mineral transport, and hormone regulation factors have changed the understanding of calcium and phosphate homeostasis in the fetus and the newborn. This article will guide clinicians through an updated review of calcium and phosphate physiology, then review specific conditions pertinent to successful neonatal care. Furthermore, with the advancement of increasingly rapid molecular genetic testing, genomics will continue to play a greater role in this area of fetal diagnostics and prognostication.

Keywords: calcium, phosphate, magnesium, vitamin D, PTH, genetics, fibroblast growth factor 23

TAKE HOME POINTS:

1. Fetal and neonatal mineral metabolism differs significantly from that in later life.
2. The regulation of sodium/phosphate cotransporter activity in the renal tubules is the primary mechanism by which phosphate homeostasis is maintained. Major phosphaturic hormones that regulate renal phosphate handling are PTH and FGF23.
3. Advances in genetics have identified new gene mutations in which have clarified the causes of several conditions previously thought to be "idiopathic."
4. A thorough understanding of the topic is essential to correct diagnosis and treatment of disorders of calcium and phosphate in the newborn.

INTRODUCTION

Over the past 35 years there have been significant advances in the understanding of materno-fetal mineral homeostatic mechanisms. Parathyroid hormone related peptide (PTHrP) was first described in 1985 as a new compound with parathyroid hormone (PTH)-like bioactivity that accounted for the discrepancy between human umbilical cord and maternal PTH levels (1). This discovery provided new insight as to why fetal PTH levels were so low, yet fetal calcium levels were maintained higher than and independent of maternal calcium concentrations. Another important novel finding was made in 2000, when bone-derived hormone Fibroblast Growth Factor-23 (FGF23) was found to cause autosomal dominant hypophosphataemic rickets (ADHR), which provided the underlying mechanism for the previously unknown "phosphaturic factor" causing hypophosphataemia (2, 3). Genomic discoveries have continued to provide new insights into the mechanisms facilitating transplacental bone mineral transport and unveil the causation of conditions previously thought to be idiopathic. As the fetus accumulates 80% of its bone mineral content in the third gestational trimester (4), this time is critical to achieve normal skeletal mineralisation by 40 weeks gestation and support successful transition to post-natal life. Passive and active transport of bone-minerals occurs across the placenta to achieve higher fetal concentration of calcium, phosphate, and magnesium compared to maternal levels. Once the baby is born, loss of placental delivery of minerals causes a sudden drop in serum concentrations of these bone minerals which triggers a rise in regulating factors such as PTH, 1,25-dihydroxyvitamin D [$1,25(OH)_2D$, calcitriol] and FGF23 to maintain postnatal homeostasis. This article will first examine current understanding of fetal-to-neonatal mineral homeostasis mechanisms, and then review specific conditions pertinent to successful neonatal care. Magnesium and vitamin D homeostasis will also be briefly discussed.

Fetal Calcium Homeostasis

Fetal blood calcium concentrations are maintained ∼0.3–0.5 mmol/L higher than in maternal circulation, with the placenta transporting 100–150 mg/kg/day of calcium during the third trimester (4–6). To achieve this, active materno-fetal transplacental transport is facilitated by transmembrane calcium-selective channel TRPV6, calbindin D_{9k} and plasma membrane calcium-ATPase. Once calcium has been delivered to the fetus, concentrations are tightly regulated by the calcium sensing receptor (CaSR) which is primarily expressed in the fetal parathyroid glands and kidneys. The CaSR activates magnesium-dependent G-protein coupled downstream signalling cascades to control PTH secretion and renal calcium handling. Mutations in *CASR* result in distinct phenotypes causing either hyper- or hypocalcaemia.

PTH is integral for achieving normal bone mineralisation and maintaining fetal calcium homeostasis by regulating expression of calciotropic genes and other solute transporters within the placenta (7). By the 10th week of gestation PTH is synthesised from fetal parathyroid glands, but circulating concentrations are kept low during fetal life due to relative hypercalcaemia dictated by the CaSR. Fetal parathyroid glands differentiate from endoderm cells in the third and fourth pharyngeal pouches, and mutations in any of the involved genes or transcription factors results in several genetic hypoparathyroidism conditions (8, 9). Both PTH and PTHrP, acting on PTH1 receptor to increase resorption of calcium from bone and kidney and expression of 1a-hydroxylase enzyme, play a critical role in endochrondral bone formation and stimulation of placental calcium transport (4, 10, 11).

Birth causes disruption of the maternal-fetal calcium supply and rapid 30% drop in serum calcium concentrations (4). This triggers a 2–5-fold increase in PTH secretion to stimulate calcitriol synthesis, resorption of calcium from renal tubules, and mobilisation of calcium from skeletal stores to maintain normocalcaemia in the first 48 postnatal hours (4, 6). Hypocalcaemia can be much more pronounced in premature infants due to lack of third trimester bone mineral accretion and gestational unresponsiveness of the parathyroid glands (6). The gastrointestinal tract then becomes the main source of calcium for the newborn. As such, the feeding mode and volume determines calcium availability. For example, exclusively breast-fed infants receive ∼200 mg/day calcium (12). Active calcium absorption is driven by calcitriol. The PTH surge drives upregulation of calcitriol synthesis which increases serum total calcium to adult values within 48 h which are then strictly maintained between 2.12 and 2.62 mmol/L (12). There is evidence, however, that the newborn gut is not fully responsive to calcitriol until 4 weeks of age (4).

Fetal Phosphorus Homeostasis

Fetal serum phosphate is maintained ∼0.5 mmol/L higher in the fetus compared to the mother, though the mechanisms for placental transport are unclear (4). The majority of phosphate, ∼60–70 mg/kg/day, is also accumulated during the third trimester of gestation and is stored primarily within bone as hydroxyapatite (5, 6). Phosphate is integral to endochondral bone formation by mediating hypertrophic chondrocyte apoptosis, and in the mineralisation of fetal bone as it is incorporated into the osteoid allowing calcium to bind to it (13).

The homeostatic mechanisms by which phosphate concentrations are "sensed" in humans are not fully understood, though it is possibly via a plasma membrane complex and that intestinal lumen levels are involved in feedback-regulation on renal phosphate reabsorption (14, 15). Elevation of extracellular phosphate activates FGF23, the primary endocrine regulator of phosphate that is produced by osteocytes and osteoblasts in bone. A complex signalling cascade is then activated when FGF23 binds with co-receptor Klotho to the FGF-receptor (FGFR1) in the kidney (16). The three primary actions of FGF23 are: promoting phosphaturia by phosphorylation of the sodium/hydrogen exchange regulatory factor (NHERF1 coded by *SLC9A3R1*) and down-regulation of NaPi type 2a/2c co-transporters in the renal proximal tubule (coded by *SLC34A1* and *SLC34A3,* respectively); reducing calcitriol metabolism by downregulation of 1-alpha-hydroxylase activity and upregulating catabolic enzyme 24-hydroxylase activity; and have a direct effect

on parathyroid glands to reduce PTH secretion (14–17). Mutations in any of these genes can cause variable degrees of nephrocalcinosis, hypophosphataemia, hypercalcaemia, and rickets (18–20). There is emerging evidence that phosphate also has a direct effect on the parathyroid glands and CaSR, in that hyperphosphataemia directly inhibits CaSR activity which, in turn, stimulates PTH secretion and thus promotes renal phosphate wasting from the proximal renal tubule (21).

Immediately after birth phosphate concentrations are low, ∼2.6 mmol/L, and rise during the first 48 h of life (6). This rise is likely to be due to immature renal excretion mechanisms (4). The main source of phosphate is dietary, so the method of infant feeding will determine phosphate loading. After birth, normal concentrations of phosphate are dependent on growth and must be interpreted within the context of age-related laboratory reference ranges.

Fetal Magnesium Homeostasis

Like calcium and phosphate, fetal magnesium concentrations *in utero* are also maintained independently of maternal concentrations, only 0.05 mmol/L higher though, and accrual of ∼3–5 mg/kg/day primarily occurs in the third trimester gestation (4, 6, 22). Magnesium is absorbed via TRPM6 and TRPM7 transcellular transporters in the gut and renal tubules, however, and the precise mechanisms controlling placental magnesium transfer and fetal homeostasis remain unknown (4). Magnesium is an important cation that binds to the CaSR, causing modest influence on PTH secretion, and hypomagnesemia can blunt effective PTH secretion (23, 24).

Fetal Vitamin D Homeostasis

Vitamin D plays a much more important role in postnatal life rather than assisting transplacental mineral homeostasis or fetal mineral accretion. Whilst maternal 25-hydroxyvitamin D readily crosses the placenta to achieve fetal concentrations that are 75–100% of maternal concentrations (1), maternal calcitriol does not cross the placenta but is synthesised primarily in fetal kidneys to achieve fetal concentrations 50% that of maternal (4). These low concentrations are likely suppressed by the elevated fetal serum calcium and phosphate, and low concentrations of PTH. However, it has been demonstrated in animal models that the $1,25(OH)_2D$ vitamin D receptor (VDR), and thus by proxy calcitriol, is not actually required *in utero* for the fetus to achieve normal calcium, phosphorus, or PTH homeostasis, or for normal skeletal mineralisation as the placenta is providing the required mineral transfer (25, 26). It is after birth that the role of calcitriol becomes vital.

After birth the level of vitamin D (cholecalciferol) intake depends on the mode of feeding as the gut becomes the main source of absorption. Breast milk primarily contains vitamin D in the form of cholecalciferol, as very little 25(OH) vitamin D passes from maternal serum into breast milk. Whilst there is good correlation between maternal cholecalciferol intake and infant serum 25(OH) vitamin D concentrations (27), breast milk contains only a small amount of cholecalciferol – no more than 25 IU/L – which is insufficient to meet newborn daily requirements (28, 29). Though infant formulae are fortified with vitamin D, it is the international consensus guidance that all infants should be supplemented with cholecalciferol 400 IU/day for every infant until 12 months of age regardless of feeding mode (30).

TABLE 1 | Summary of the various causes of neonatal hypocalcaemia.

Early hypocalcaemia <72 h life	Late hypocalcaemia >72 h life to 10 days of life inclusive

• Prematurity
• Intrauterine growth restriction
• Sepsis
• Perinatal asphyxia causing cellular damage and release of intracellular phosphate
• Iatrogenic:
  ○ Transfusions with citrate blood products
  ○ Lipid infusions
  ○ Loop diuretics
• Maternal factors:
  ○ Severe Vitamin D deficiency
  ○ Pre-eclampsia
  ○ Gestational diabetes associated with hypomagnesaemia
  ○ Hyperparathyroidism suppressing infant PTH synthesis
  ○ Anti-convulsants
  ○ High dose antacids
	• Vitamin D deficiency due to inadequate synthesis, intake or absorption
	• Increased phosphate load:
	  ○ Enteral feeding with cow's milk
	  ○ Parenteral nutrition
	• Primary Hypoparathyroidism:
	  ○ Isolated vs. Syndromic
	• Secondary Hypoparathyroidism:
	  ○ Pseudohypoparathyroidism due to renal disease or *GNAS* mutations
	  ○ Congenital heart disease
	  ○ Renal disease
	  ○ Gastrointestinal disease
	  ○ Critical illness
	• Osteopetrosis

NEONATAL HYPOCALCAEMIA

Neonatal hypocalcaemia is defined in two ways: one, in term and pre-term infants with birth weight >1,500 g as total serum calcium <2.0 mmol/L or ionised calcium <1.1 mmol/L; and two, in pre-term infants with low birth weight (LBW) <1,500 g as a total serum calcium <1.75 mmol/L or ionised calcium <1 mmol/L (31). Clinical signs of hypocalcaemia are difficult to elicit in newborns and hypocalcaemia is often asymptomatic within 72 h of birth. Acute hypocalcaemia can present as apnoea, irritability, jitteriness, muscle cramps, tetany (including laryngospasm), seizures, cardiac arrhythmias, and QT-segment prolongation. Chronic hypocalcaemia can be more subtle, presenting with dental enamel hypoplasia, subcapsular cataracts, cardiomyopathy, congestive cardiac failure, and basal ganglia calcifications.

The causes of neonatal hypocalcaemia are summarised in **Table 1**. Parathyroid glands can take >48 h to become responsive to the fetal-to-neonatal transition and important causes of hypocalcaemia can be helpfully thought of as early onset (<72 postnatal hours), or late onset (>72 postnatal hours) (31–33). Several genetic mutations have been found to cause Primary Hypoparathyroidism and should be considered when hypocalcaemia lasts >72 h. Isolated causes of hypoparathyroidism include *GMC2* or PTH-gene mutations, autosomal dominant activating *CASR* or *GNA11* mutations, and X-linked *SOX3* mutations (8). Mutations in *CASR* result

in distinct phenotypes causing either hyper- or hypocalcaemia. Activating (gain-of-function) *CASR* mutations decrease the set-point of CaSR and PTH is not secreted when low calcium levels would normally trigger PTH release, resulting in Autosomal Dominant Hypocalcaemia (ADH). Clinically this presents as hypocalcaemia associated with an inappropriately normal-high urinary calcium excretion, presumably due to increased activity of the CaSR in the kidney.

Hypoparathyroidism can also be associated with more complex syndromes including: 22q Deletion Syndrome; CHARGE association (*CHD7*); Autoimmune polyglandular syndrome type 1 (*AIRE*); Hypoparathyroidism, sensorineural deafness, and renal dysplasia (HDR) syndrome (*GATA3*); mitochondrial disorders; Sanjad-Sakati and Kenney-Caffey syndromes (*TBCE* or *FAM111A*) (34). The most prevalent of these complex syndromes is DiGeorge syndrome (DGS) due to deletions in the chromosome region 22q11 involving the candidate gene *TBX1,* however microdeletions on chromosome 10 (*GATA3* or *NEBL*) may cause similar phenotypes also associated with cardiac abnormalities (35). Hypocalcaemia in DGS is usually transient and due to underdeveloped parathyroid glands, occurring in 60% of patients mostly during the neonatal period (36). Even normocalcaemic DGS infants are likely to have serum calcium concentrations in the lower half of the normal range, so all should be routinely screened for hypocalcaemia (37).

Osteopetrosis is a very rare cause of neonatal hypocalcaemia, where defective osteoclasts are unable to remodel bone. Hypocalcaemic tetany and seizures can be a presenting feature due to the inability to mobilise calcium stores from bone (38). Osteopetrosis is also associated with increased bone mass, fragility fractures, and bone marrow failure (39). It is usually identifiable with plain radiograph with osteosclerosis and "bone-within-bone" appearance on skeletal survey.

NEONATAL HYPERCALCAEMIA

Whilst there is no consensus on definition, neonatal hypercalcaemia may be considered when calcium is greater than two standard deviations above the normal mean (ionised calcium above 1.32 mmol/L or adjusted serum calcium >2.6 mmol/L) (40), or a total serum calcium >2.9 mmol/L (41). Clinical features in the newborn can be difficult to identify and may include polyuria, polydipsia, lethargy, vomiting, abdominal pain, failure to thrive, irritability, and seizures. The causes of hypercalcaemia associated with appropriately suppressed PTH secretion are extensive. Neonatal sepsis is the most common cause that lasts longer than two consecutive days, possibly due to extra-renal macrophage production of calcitriol and/or increased cytokine activity (41). Other important common causes include: subcutaneous fat necrosis where granulomatous inflammatory cells express increased calcitriol; increased calcium and phosphate intake in infants receiving parenteral nutrition; Vitamin D intoxication; and Williams-Beuren Syndrome due to gene deletion on chromosome 7q11.23, which classically present with mild hypercalcaemia (2.9 mmol/L) associated with supravalvar aortic stenosis and distinctive facial features (42).

There has been a number of new genetic discoveries for various causes of neonatal hypercalcaemia. Identification of the active calcium placental transport mechanism, transmembrane calcium-selective channel TRPV6, provides genetic explanation for a condition previously labelled "Transient Neonatal Hyperparathyroidism," which was thought to be have been caused by congenital vitamin D deficiency. Newly identified compound heterozygous missense mutations in *TRPV6* were found to prevent adequate transplacental calcium transport and cause potentially lethal skeletal abnormalities (undermineralised bone, fractures, periosteal, and metaphyseal changes), elevated PTH, hypomagnesaemia and hypovitaminosis D (33, 43–45). New genetic mutations in vitamin D metabolism and urinary phosphate excretion have also been identified as causes of previously labelled "Idiopathic Infantile Hypercalcaemia." Loss-of-function mutation in *CYP24A1* [encoding vitamin D breakdown enzyme 25(OH) vitamin D_3 24-hydroxylase] and in *SLC34A1* (encoding renal proximal tubular NaPi co-transporter) and NHERF1 (a modifier of *SLC34A1*) cause accumulation of calcitriol, hypercalcaemia, hypercalciuria, and nephrocalcinosis (19, 46). An awareness of these last two conditions is important in reducing long-term kidney disease in adulthood. Other causes of neonatal hypercalcaemia currently do not have an identifiable cause and are truly idiopathic.

When hypercalcaemia is associated with inappropriately detectable PTH (within laboratory "normal" range or elevated), then causes to consider include rare inactivating (loss-of-function) *CASR* gene mutations which result in CaSR insensitivity and thus PTH secretion is not switched-off until higher-than-normal calcium concentrations, causing Familial Hypocalciuric Hypercalcaemia (FHH). Clinically this presents as generally asymptomatic hypercalcaemia, inappropriately detectable concentrations of PTH, associated with reduced renal calcium excretion. The mode of inheritance appears to cause a "dosage effect" with regard to the severity of hypercalcaemia (47). In contrast, homozygous or compound heterozygous loss-of-function *CASR* mutations, or heterozygous mutations where the mother is not affected, cause Neonatal Severe Hyperparathyroidism (NSHPT), which is a severe phenotype that is associated with life-threatening hypercalcaemia, hyperparathyroid bone disease and multiple fractures. Early diagnosis is critical to prevent death or neuromotor delay (48).

RICKETS AND RICKETS-LIKE DISORDERS

Rickets is a disorder of the growth plate resulting from defective chondrocyte apoptosis and osteoid mineralisation. Rickets can be sub-classified as: calciopenic (due to dietary deficiency of vitamin D or calcium, or due to defects of vitamin D metabolism or action); or phosphopenic (due to renal phosphate wasting or deficiency of phosphate intake). A concurrent serum PTH measurement can be useful when distinguishing between calciopenic (PTH should be elevated) and phosphopenic rickets (PTH likely within normal laboratory reference range or only modest elevation). The radiological and clinical features depend on the child's age at presentation and underlying cause, but

include metaphyseal widening, under-mineralised bone matrix (osteomalacia), delayed closure of fontanelles, softening of the skull bones (craniotabes), parietal and frontal bone bossing, craniosynostosis, bowing of long bones, pseudofracture (Looser zones) and fractures, sequelae of hypocalcaemia (seizures, tetany, dilated cardiomyopathy), failure to thrive, decreased muscle tone, and delayed motor milestones (30, 48, 49). It is important to note that craniotabes and ulnar cupping can be normal variants seen in healthy neonates without correlation to maternal or neonatal vitamin D concentrations (4).

Nutritional rickets are the most common form of rickets in the newborn period, and consensus guidelines recommend vitamin D sufficiency of 25(OH) vitamin D >50 nmol/L (30). Breastfed infants of vitamin D deficient mothers, especially with darker skin pigmentation, are high risk and should be routinely screened. The resulting hypocalcaemia is exacerbated by an immature newborn PTH-response. There is significant controversy about the appropriateness of the term "congenital rickets," as mothers were found to have compounding conditions (such as malnutrition or malabsorption) that interfered with vitamin D metabolism. Therefore, "neonatal rickets" is the preferred terminology (4). Genetic mutations in vitamin D synthesis (25-hydroxylase and 1-alpha-hydroxylase deficiencies) or action (VDR mutations) are rarer causes of vitamin D associated rickets. The demand for skeletal mineral delivery is high, especially in preterm babies, and rachitic skeletal changes that are absent at birth can develop rapidly within 16 days after delivery (50).

Hypophosphataemic rickets uncommonly presents in the newborn. Special consideration is needed, however, to ensure adequate enteral or parenteral supplementation meets the increased skeletal mineralisation demands. Feeding with amino-acid elemental formulas, such as Neocate®, and high-dose antacids, has been associated with reduced bioavailability of phosphate resulting in hypophosphataemic rickets and fractures (51, 52). Hemizygous mutations in phosphate-regulating endopeptidase (*PHEX*) gene lead to overexpression of FGF23 and cause X-Linked hypophosphataemic rickets (XLH) (53). While the renal phosphate wasting is present from birth, XLH does not tend to become clinically apparent until child begins to weight-bear.

The differential diagnosis for rachitic-appearing skeletal changes in newborns is large and includes neonatal hyperparathyroidism, skeletal dysplasias, hypophosphatasia, metaphyseal chondrodysplasia, osteogenesis imperfecta (OI), and vitamin C deficiency (Scurvy) (49). Of these, hypophosphatasia caused by loss-of-function *TNSALP* mutations, in its severest forms (perinatal and infantile) can present with profound skeletal hypomineralisation and bone deformity, hypercalcaemia with downregulation of PTH, and hypercalciuria (54).

BONE FRAGILITY

When considering conditions that present *in utero* and the neonatal period with bone fragility, OI and OI-like disorders may jump to mind. However, they do not tend to present with biochemical mineral disturbance. The most likely cause of fragile or poorly mineralised bones associated with mineral disturbance in the newborn is Metabolic Bone Disease of Prematurity (MBDP), which has multiple contributors to its characteristic biochemical and radiological findings, see **Table 2**. Clinical features can develop between 3 and 12 weeks of age, so it is important to screen routinely for biochemical evidence of MBDP with serum alkaline phosphatase (ALP), albumin-adjusted calcium, phosphate, and PTH concentrations from 4 weeks of age in at-risk groups (5, 55). In the premature infant gut phosphate is more readily absorbed than calcium so it is important to ascertain whether MBDP is due to hypophosphatemia or hypocalcaemia. A PTH level paired with serum calcium and phosphate, and urine renal tubular resorption of phosphate (TRP) measurement will help differentiate between hypophosphatemia or hypocalcaemia. Secondary elevation of PTH will occur to maintain normocalcaemia, whereas this compensation does not tend to occur with hypophosphatemia and is associated with decreased phosphaturia (56). Initiating the correct supplementation depending on deficiency is important, as phosphate supplementation in the hypocalcaemic state will bind ionised calcium, exacerbating hypocalcaemia, driving PTH higher, exacerbating renal phosphate loss, and worsening MBPD. While there is no biochemical cut-off consensus to diagnose MBDP, in preterm infants <33 weeks of gestation the combination of bone turnover marker ALP >900 IU/L and phosphate <1.8 mmol/L is associated with sensitivity of 70% and specificity of 100% of having low bone mineral density at 3 months corrected age (57). In practice, lower threshold ALP 500–800 IU/L in infants <34 weeks of gestation is used to implement supplementation (58, 59).

Radiological changes occur late as bones will only appear generally osteopenic on X-ray when >20% of bone mineral is lost (60). Fragility fractures can occur with incidence reported between 17 and 34%, usually after 10 weeks of age in long bones or ribs up until 6 months of uncorrected gestational age (5). Although all fractures are painful, rib fractures often remain undetected by parents and clinical staff until found incidentally on routine chest X-ray (61). Routine screening with X-ray for

TABLE 2 | Factors contributing to Metabolic Bone Disease of Prematurity.

Antenatal factors	Postnatal factors
• Prematurity <34 weeks of gestation • LBW <1,500 grams • Pathological condition inhibiting macro- and micronutrient placental transfer (chorioamnionitis, pre-eclampsia, intrauterine growth restriction)	• Necrotising enterocolitis • Liver or Renal disease • Late establishment of enteral feeds/prolonged total PN >4 weeks • Chronic lung disease/ bronchopulmonary dysplasia • Medications causing bone resorption (loop diuretics, glucocorticoids) • Antacids • Methylxanthines (caffeine for apnoea of prematurity)

MBDP is not indicated in the absence of biochemical disease. Techniques such as dual energy X-ray absorptiometry, peripheral quantitative computed tomography, and quantitative ultrasound have been utilised in research settings (60). There are, however, no normative data <5 years of age, which limits their widespread clinical use.

Prevention of MBDP is limited by multiple factors including the inability of premature infants to tolerate full enteral feeding volumes, the inability to deliver high-dose calcium and phosphate via parenteral nutrition (PN) due to solubility and precipitation limits, and reduced gut absorption of calcium and phosphate. The recommended range delivered via PN route is calcium 40–120 mg/kg/day (1.3–3 mmol/kg/day) and phosphate 31–71 mg/kg/day (1–2.3 mmol/kg/day), and via enteral route is calcium 120–200 mg/kg/day and phosphate 60–140 mg/kg/day (62, 63). Enteral calcium and phosphate supplementation should not be given simultaneously or with milk-meals, to avoid precipitation (56). Effective treatment of MBDP should include routine supplementation with cholecalciferol once on full enteral feeding, aiming for serum 25(OH) vitamin D >50 nmol/L (56, 58). Once treatment of MBDP has been initiated ongoing monitoring of ALP, albumin-adjusted calcium, phosphate, serum creatinine levels, and urine creatinine and TRP is important to avoid hypercalcaemia, hyperphosphataemia, and nephrocalcinosis.

Investigations to Arrange

Essential investigations to assess calcium and phosphate homeostasis include concurrent serum calcium, phosphate, magnesium, PTH, albumin, ALP, electrolytes and renal function, and 25-hydroxyvitamin D levels. Most laboratories provide a calcium corrected for albumin concentration, if not then the following formula will give you the albumin-adjusted serum calcium mmol/L: measured total serum calcium mmol/L + 0.02 × (40 gm/L – measured serum albumin gm/L). High risk infants such as LBW, prematurity <34 weeks, infant of diabetic mother, and prenatal asphyxia should be routinely screened for hypocalcaemia within the first 48 h of age.

If neonatal blood quantities are particularly scarce, then a capillary or venous blood gas will give an ionised calcium value, and serum phosphate can be used as an indirect marker of PTH activity (i.e., low phosphate concentrations reflect high PTH activity and vice versa). Additional investigations such as $1,25(OH)_2D$ and serum DNA for genetic analysis may be required but this should follow discussion with local Paediatric Endocrinology service.

Urinary electrolytes and glucose should be part of routine analysis, to calculate renal calcium: creatinine ratio, and to assess renal tubular function.

Radiology should be considered in specific circumstances, namely when bone mineralisation or skeletal dysplasia is a concern. Usually, diagnosis can be made on a limited series to reduce the neonate's radiation exposure including plain films of anterior-posterior chest and metaphysis of a long bone (e.g., unilateral distal femur or wrist). Specific skull films (looking for Wormian bones) or a full skeletal survey are required if bone fragility or skeletal dysplasia are a concern. The need for these more extensive investigations should be discussed with a local specialist paediatric Radiologist.

TREATMENT

Appropriate treatment depends on the cause. Where the primary cause is mineral deficiency, additional supplements of calcium, phosphate and, if necessary, magnesium should be given. This can usually be achieved orally but, if demineralisation is severe then intravenous infusion may be required. Vitamin D deficiency should always be corrected.

Hypoparathyroidism, particularly if symptomatic, may require treatment with vitamin D analogues calcitriol or its prohormone alfacalcidol, but caution must be taken to ensure that hypercalciuria and nephrocalcinosis do not result from this treatment. New treatment options in the form of subcutaneous injections of synthetic human PTH teriparatide (hPTH 1-34) and recombinant human PTH (rhPTH 1-84) have been used, particularly where activating mutations of CaSR are the cause of the hypoparathyroidism (23, 64, 65). Calcilytic agents, which reduce the sensitivity of CaSR, are also being investigated as novel therapies for activating CaSR mutations (66).

Hyperparathyroidism can sometimes be corrected with the use of bisphosphonate therapy (although this can lead to an increase in PTH secretion) and/or calcimimetic agents such as cinacalcet (which activate the CaSR, thus increasing the receptor's sensitivity and reducing PTH secretion). Total parathyroidectomy may occasionally be required for intractable NSHPT. Burosumab, a monoclonal antibody to FGF23, is not yet licensed for infants with XLH under 1 year of age but is available in the United Kingdom under an Early Access to Medicines scheme.

ROLE OF GENOMICS

Genetics plays an important part in diagnosis as an increasing proportion of cases have been found to have a genetic basis. Liaison with a clinical geneticist can be invaluable.

CONCLUSION

The physiology of mineral metabolism differs considerably between fetal and post-natal life. The neonatal period is one of transition from one to the other and a thorough understanding of these processes is required to be able to diagnose and treat the various conditions when they arise.

AUTHOR CONTRIBUTIONS

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Neonatal Bone Disorders

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Neonatologists care for newborns with either an antenatal suspicion or postnatal diagnosis of bone disease. With improved ultrasound imaging techniques, more cases of neonatal bone disorders are identified antenatally and this requires further diagnostic/molecular testing either antenatally or soon after birth for confirmation of the diagnosis and facilitating subsequent management. Prompt diagnosis is vital in certain conditions where initiation of treatment is time critical and life saving. We outline an approach to diagnosis, investigation, and management of a neonate with a suspected bone disorder.

Keywords: newborn, bone, mineralization, structural, antenatal

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INTRODUCTION

The fetus accrues 80% of bone mineral content between 24 weeks gestation and term (1). Neonatal bone disorders encompass a spectrum of bone conditions resulting from either structural or mineralization defects, a process that has already commenced in the fetus (2). Good quality fetal ultrasound images are likely to detect bone abnormalities in the second trimester onwards (3). This is followed by molecular confirmation of the diagnosis in some cases and postnatally in the rest. Early diagnosis and effective management can be vital to the impact this can have on childhood, adolescent, and adult bone heath. In this manuscript, we have outlined an approach to diagnosis, investigation, and management of neonatal bone disorders.

APPROACH TO NEONATAL BONE DISORDERS

Fetal ultrasonography has been used since the 1950's to estimate gestational age, detect multiple pregnancies and diagnose fetal anomalies (4). Occasionally antenatal ultrasound evaluation raises suspicion of a bone disorder in the fetus (5). For example, severe osteogenesis imperfecta can be diagnosed antenatally based on multiple long bone fractures and limb deformities. Also fetal femora or humeri length of less than the 5th centile or −2 SD from the mean in a second trimester scan often raises the suspicion of skeletal dysplasia (6). However more often antenatal fetal radiographs show a constellation of features which may be common to several disorders. Equally, in many cases, antenatal imaging may have been completely normal. Based on postnatal x-ray findings and biochemistry, bone disorders can be largely categorized into either a predominantly structural bone defect (with normal bone biochemistry) or a mineralization bone defect (with abnormal bone biochemistry). This classification aids further targeted investigations.

STRUCTURAL BONE DEFECTS

Osteogenesis Imperfecta

Osteogenesis Imperfecta (OI) is a genetic disorder of increased bone fragility and low bone mass which has a wide spectrum of severity. It has an incidence of 1 in 10–20,000 births and occurs with equal frequency in genders and ethnic groups.

The majority are due to defects in the amount or quality of Type 1 collagen which is coded for by two genes, COL1A1 and COL1A2. These are autosomal dominant in inheritance accounting for ∼90% of cases of OI. Autosomal recessive forms have been recognized in the past 20 years and account for between 5 and 10% of cases. These are due to a variety of different genes that predominantly affect the synthesis of Type 1 collagen.

The traditional classification of OI described four types with Type I the mildest and most frequent form, Type III a severe form with multiple fractures and bowing deformity of limbs and Type IV an intermediate form of moderate severity (7). These three types are associated with long term survival. An additional form, Type II was described, also known as perinatal lethal as affected babies would die in the neonatal period due to respiratory insufficiency. As a consequence of the discovery of new forms of OI in recent years, revised classifications have been proposed (8, 9). However most specialists who manage affected individuals like to categorize them as mild, moderate or severely affected.

Clinical Presentation

There are several ways in which a baby with OI may present in the neonatal period.

Severe Forms

Antenatal detection at the time of routine ultrasound scans is increasingly identifying severe forms *in utero*. Skeletal abnormalities such as bowed limbs, fractures, and small chest size may lead to a precise diagnosis of OI or a broader description as a lethal skeletal dysplasia which is only recognized as OI after birth.

If not identified in the antenatal period, some severely affected infants with small deformed chests due to the presence of multiple rib fractures (**Figures 1-1a**) will develop respiratory insufficiency within a few days of birth requiring assistance ranging from supplemental oxygen to positive pressure ventilation. More commonly, a severely affected baby will present at birth with evidence of short stature, bowed limbs and multiple fractures (**Figures 1-1b**). Subsequent investigation with reviews of skeletal surveys by experienced clinicians will usually lead to a diagnosis of OI which can be confirmed by genetic testing.

Mild and Moderate Forms

These may present in the neonatal period in several ways. There may be evidence of a short bowed femur which might have been detected on an antenatal scan. It is often not apparent that this is due to OI until the child starts to fracture, which may not be for some months or years after birth. An alternative presentation is with a dislocated or unstable hip due to the ligamentous laxity known to be characteristic of OI. An occasional, but uncommon, presentation is when an affected baby presents in the neonatal period with a long bone fracture, which leads to the identification of other fractures (including rib fractures) on skeletal survey.

Assessment

There are a number of investigations of importance when a baby presents with a suspected diagnosis of OI. It is important to perform a skeletal survey including the skull and spine with review by a pediatric radiologist looking for features such as Wormian bones (accessory skull bones completely surrounded by suture lines), rib and vertebral fractures and bowing deformity

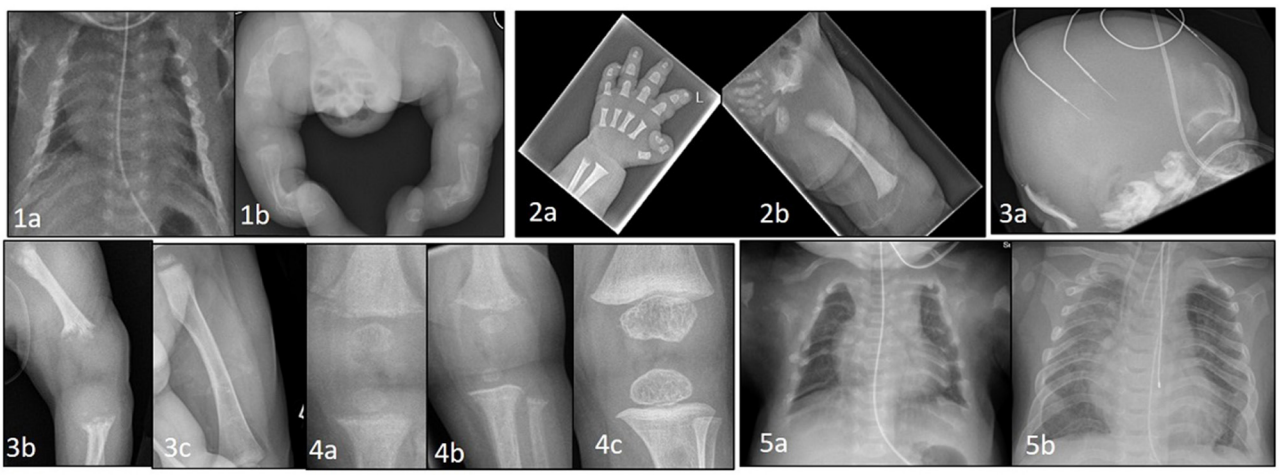

FIGURE 1 | (1a) Thin, gracile and beaded ribs in a case of severe osteogenesis imperfecta at birth. **(1b)** Lower limbs showing bowing and deformity of long bones with multiple fractures in various stages of healing. **(2a)** Hand x-ray in achondroplasia showing short metacarpals and phalanges with trident sign. **(2b)** Hip shows classic features of horizontal acetabular roof, squared off iliac crest, small sacrosciatic notch, and scalloping of proximal femur. **(3a)** Perinatal hypophosphatasia at birth showing severe skull hypomineralization. **(3b)** severe hypomineralization of long bones and metaphyseal lucencies. **(3c)** Healing of metaphyseal lesions and markedly improved mineralization after 12 months of asfotase alfa treatment. **(4a)** Knee x-ray in a case of NHSPT at 3 months showing metaphyseal irregularity, coarsening of trabeculae, and subperiosteal resorption. **(4b)** progressive changes at 6 months, and **(4c)** complete healing following total parathyroidectomy. **(5a)** Chest X-ray at birth in a baby with TRPV6 mutation. Ribs are malformed with severe demineralization. **(5b)** Spontaneous improvement at 2 years of age with remodeling, improved mineralization of ribs and thoracic volume.

of long bones. Additional imaging of the cervical spine and brain may be indicated in severely affected infants for the presence of cervical spine abnormalities which may compromise spinal cord function, or hind brain abnormalities such as basilar invagination.

All infants with OI should have blood tests to check bone profile, renal function, and vitamin D levels to ensure these are normal if bisphosphonate treatment is being considered. Severely affected babies are likely to be cared for on a neonatal unit with routine assessment of respiratory function.

Genetic testing is now readily available in many countries with initial testing for abnormalities in COL1A1 and COL1A2 which will account for 90% of cases. If the results of these are normal consideration should then be given to testing for the recessive forms of OI (10). Although the results will not be available for several weeks they are important not only for confirmation of the diagnosis but for genetic counseling to the parents for future pregnancies.

Management

A severely affected baby will be managed initially on a neonatal intensive care unit. There is no reason why milk feeds cannot be commenced if there is no significant respiratory distress. This will often be *via* a nasogastric tube initially, as a continuous feed with progression to bolus feeds if well-tolerated. Breast feeding can also be attempted if there is no significant respiratory distress. Many severely affected infants will still require nasogastric feeding at the time of hospital discharge. An important aspect of nutrition in such babies is the recognition that they are short and therefore it is not appropriate to expect their weight gain to be within the normal centiles (11). Such a practice may lead to overfeeding and excess weight gain.

The input of a multidisciplinary OI specialist team is important in management and will often be undertaken by this team visiting the neonatal unit to review the baby and meet the parents. Such support is also important for the staff of the neonatal unit who may not be familiar with managing such babies and will be uncertain of activities such as handling. The specialist OI team will educate the parents and staff about the condition with information such as likely prognosis and outcome, how to handle, dress, feed, wash, and transport the baby. They will continue as an appropriate resource to provide support until the baby is discharged and is followed up in their own specialist OI clinic. Advice about where to find additional information on OI from appropriate websites such as the Brittle Bone Society (www.brittlebone.org) in the UK will also be provided.

The use of bisphosphonate drugs (given intravenously as infusions every few months) has become an important component in the management of severely affected babies with OI in the past 20 years (12). There is evidence that bisphosphonates reduce the frequency but do not eliminate the risk of fractures. Intravenous Pamidronate has been the drug that has been most used, often given every 8 weeks in the first 2 years of life. There have been reports of the development of respiratory insufficiency in babies following the first infusion and so it is advisable to administer this whilst still on the neonatal unit (13). Hypocalcaemia is an infrequent occurrence and is more likely to occur if the baby is vitamin D deficient. Treatment of vitamin D deficiency and provision of a maintenance daily dose of at least 400 IU is important in such infants. Bisphosphonate infusions are usually given *via* peripheral veins although in some infants with difficult venous access a central line is required.

Some babies who are severely affected develop respiratory difficulty due to a small chest size secondary to pulmonary hypoplasia and in addition they can also have multiple rib fractures. Such infants would historically be considered to have perinatal lethal forms of OI and would not have survived. However, with respiratory support such as home oxygen and CPAP many of these infants are surviving and with time are capable of being weaned from such support.

It is important for parents of a new baby with OI that they receive appropriate support and information with an expectation that they will survive long term, have normal intelligence and will be able to attend a mainstream school.

Other Skeletal Dysplasias

Skeletal dysplasias, a complex group of heritable disorders of the bone and cartilage affect the fetal skeleton as it develops *in utero*. They often present as congenital bowing of the long bones, particularly the femurs, detected in the second trimester ultrasound evaluation. Short long bones when compared against normative data can determine whether there is primary rhizomelic or mesomelic shortening (6).

The common angulated femur or bent bone dysplasias in the neonatal period as described in the Skeletal dysplasia registry include Campomelic disorders (24.4%) including Campomelic and Kyphomelic dysplasias, Thanatophoric dysplasias (23.9%), OI (18.1%), short rib dysplasia (10.2%), hypophosphatasia (3.5%), Type 2 collagen disorders (3.1%), Stuve Weidman dysplasia, and Achondroplasia (1.3%) amongst others (14). Some of these dysplasias, evident on antenatal ultrasound scans are lethal in the neonatal period owing to the small chest circumference and associated pulmonary hypoplasia.

FGFR3 Chondrodysplasias

Mutations in the FGFR3 gene lead to a spectrum of conditions ranging from the lethal Thanatophoric dysplasia to the milder hypochondroplasia (15, 16). FGFR3 expressed in chondrocytes and mature osteoblasts regulates bone growth (17).

Achondroplasia

Achondroplasia is the most common form of short limb dwarfism due to a mutation in the FGFR3 gene, with a prevalence of 1 in 25,000 individuals. It is inherited in an autosomal dominant pattern with 80% arising from new spontaneous mutations (18).

Clinical Presentation

In most cases, short limbs, hands and fingers, frontal bossing, depressed nasal bridge with a large head on second trimester antenatal ultrasound scans raises suspicion of Achondroplasia. The diagnosis is subsequently confirmed by molecular testing for FGFR3 mutation. Postnatally, the diagnosis is apparent at

birth due to the rhizomelic shortening of limbs, frontal bossing, midfacial hypoplasia, and macrocephaly (19).

Management

If a diagnosis of Achondroplasia is suspected at birth, this can be confirmed by performing a skeletal survey (**Figures 1-2a,b**). Large calvaria, narrow foramen magnum, progressive reduction in the interpedicular distance in the lower spine, small trident pelvis are hallmark radiological features for Achondroplasia. MRI brain and spine is recommended to look for cervicomedullary compression occurring secondary to foramen magnum stenosis. Genetic confirmation by testing for FGFR3 mutations is also available. Bone biochemistry is often normal in these neonates (20, 21).

Treatment in Achondroplasia is mainly supportive. Some children might require neurosurgery to relieve cervical cord compression and others orthopedic intervention to help with limb deformities. Close follow up beyond the neonatal period is important with clinical assessment, history taking, and neurological examination. Because of the risk of sleep disordered breathing a sleep study is recommended in the first 6 months. With a better understanding of the molecular processes involved in Achondroplasia, various drug trials looking at blocking FGFR3 ligands, FGFR3 and its downstream signaling including tyrosine kinase inhibitors and C-type natriuretic peptide (CNP) analogs such as vosoritide, amongst others, are underway (22). Most advanced of the possible therapeutic options and the only study currently recruiting neonates, involves the use of CNP analogs in the BMN111 (Vosoritide) study by Biomarin.

BONE MINERALIZATION DEFECTS

Hypophosphatasia

Hypophosphatasia (HPP) is a rare heterogenous inherited metabolic bone disorder caused by a loss of function mutation in the ALPL gene (23), resulting in the lack of tissue non-specific alkaline phosphatase (TNSALP) activity. The more severe forms are predominantly inherited in an autosomal recessive pattern with an incidence of 1 in 100,000 live births. Deficiency in TNSALP activity results in the accumulation of inorganic pyrophosphate (PPi) which disrupts hydroxyapatite crystal formation and inhibits skeletal mineralization (24). The clinical spectrum of HPP can be variable and the two forms relevant to the neonatal period include Prenatal Benign and Perinatal Lethal form.

Clinical Presentation

Benign Prenatal HPP, as the name suggests, is a mild form of the condition with asymmetrical skeletal changes first noticed on prenatal ultrasonography, usually in the second trimester of pregnancy, including limb bowing, with or without skeletal hypomineralization and normal chest and abdominal circumference. The ultrasound appearances usually improve in the third trimester and they can run a variable postnatal clinical course ranging from the more severe infantile HPP (symptoms and signs apparent between 1 and 6 months of age) to the mild Odonto HPP where only teeth are affected with no skeletal manifestations (25, 26).

Perinatal HPP, the most lethal form, presents antenatally on fetal ultrasonography as short long bones, under mineralized skeleton, small chest, and abdominal circumference. It is apparent at birth with short deformed limbs, severely hypomineralized skeleton, small chest with hypoplastic lungs and, in some cases, pyridoxine deficiency seizures. Perinatal HPP can mimic hypoxic ischemic encephalopathy (27) and delay diagnosis in the absence of antenatal suspicion. Low serum ALP level in the presence of typical radiological features of tongue-like lucencies in the metaphysis, rickets-like changes, and hypomineralized skeleton (**Figures 1-3a–c**) should clinch the diagnosis of HPP (28).

Management

If HPP is suspected, serum ALP activity, plasma PLP, urine phosphoethanolamine levels and genetics for ALPL gene mutation alongside skeletal survey must be performed to confirm the diagnosis. Age- and sex- specific ALP activity should be used to prevent delay in the diagnosis of HPP. Prompt referral to the tertiary pediatric bone service is vital for further assessments and initiation of enzyme replacement therapy, Asfotase Alfa (Strensiq), which is time critical and life saving (28). Strensiq is delivered by subcutaneous injections three times a week for life. Follow up by a multidisciplinary team is also extremely important.

Neonatal Hyperparathyroidism

Normally parathyroid hormone (PTH) secretion from the parathyroid gland is regulated to maintain serum calcium levels within the normal range. Any drop in calcium level is sensed by the G protein coupled calcium sensing receptor (CaSR) situated on the chief cells, resulting in increased PTH secretion. The reverse occurs in hypercalcemia.

Neonatal severe primary hyperparathyroidism (NSHPT) is a result of almost complete loss of parathyroid calcium sensing due to homozygous CaSR mutations, resulting in very high serum calcium with unsuppressed PTH levels. This is a rare autosomal recessive disorder which is potentially lethal, often occurring when there is consanguinity.

Clinical Presentation and Management

Newborns may be asymptomatic at birth, but present within days to weeks. The presentation may be delayed up to 6 months when failure to thrive, poor feeding, and hypotonia (29) become apparent. They have severe hypercalcemia (commonly >4 mmol/L) (30) and X-rays show demineralized bones, subperiosteal resorption, rib fractures, and changes of rickets (**Figures 1-4a–c**). In the majority of cases hypercalcemia is severe and total parathyroidectomy is the only definitive therapy. Bisphosphonates such as pamidronate are used in the short term to control hypercalcemia until total parathyroidectomy can be performed.

Mucolipidosis Type II (i-cell disease)

This is an autosomal recessive condition caused by mutations in the GNPTAB gene that code for N-acetylglucosamine phosphotransferase complex which catalyze the post-translational modification of lysosomal enzymes. Impaired calcium transfer during pregnancy due to affected placenta is one of the postulated mechanisms for the metabolic bone disease seen in these babies. Imbalance between osteocyte and osteoclast function has been shown in mouse models (31).

Clinical Presentation and Management

Antenatal short femurs and intrauterine growth retardation have been described with radiological abnormalities detectable by 18–20 weeks of pregnancy (32). Newborns

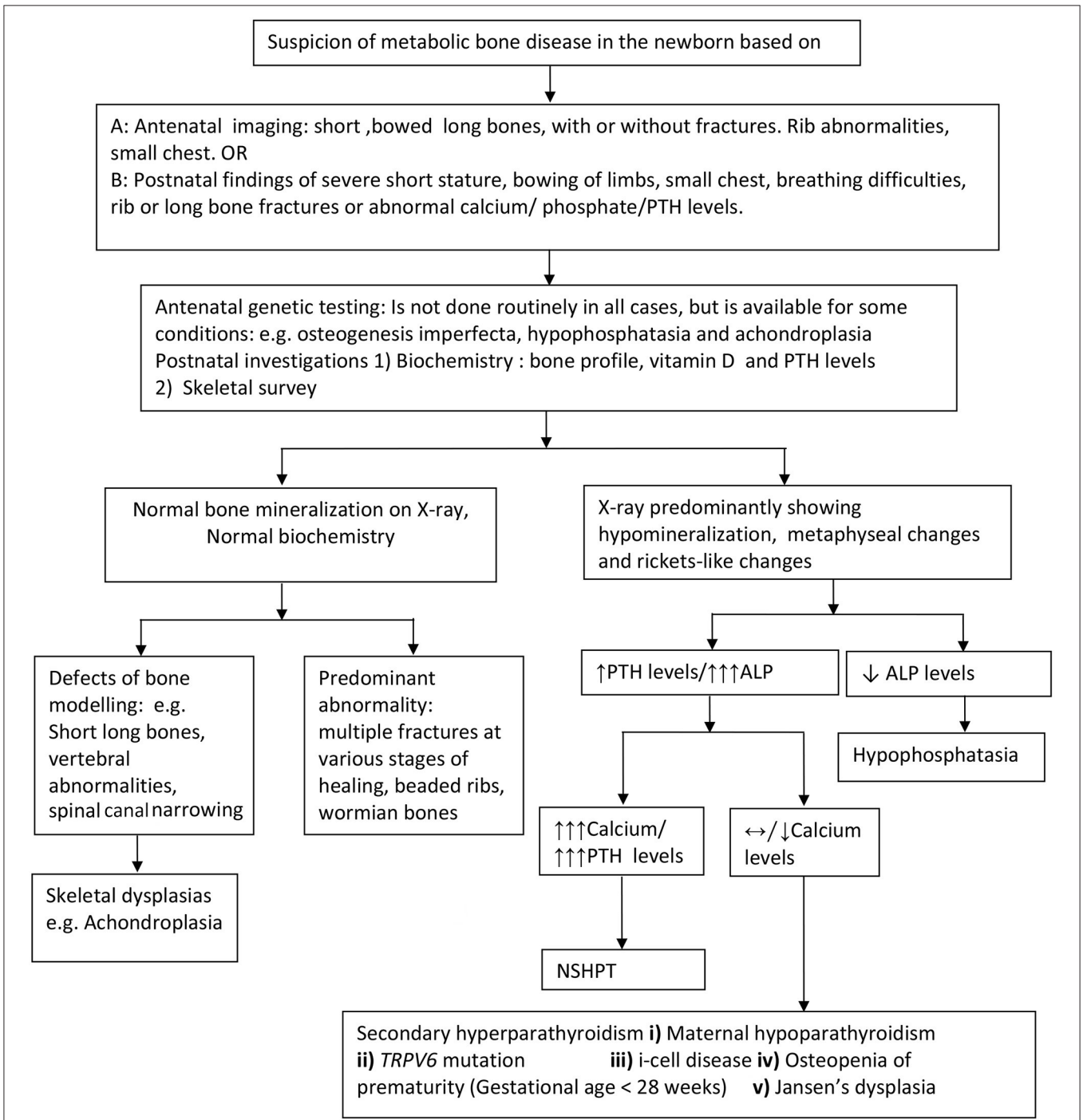

FIGURE 2 | Approach to the diagnosis of metabolic bone disorders in the newborn. PTH, parathyroid hormone levels; ALP, Alkaline phosphatase; NSHPT, neonatal severe hyperparathyroidism; TRPV6, transient receptor potential cation channel, subfamily V, member 6.

may manifest respiratory distress soon after birth (33, 34), hyperparathyroidism and fractures. X-rays show widespread osteopenia, sub-periosteal bone resorption, metaphyseal changes, shortening and undermodelling of long bones consistent with hyperparathyroidism. Biochemistry is characterized by elevated PTH and alkaline phosphatase levels with low or normal calcium levels. Diagnosis is confirmed by enzyme analysis and genetic studies for *GNPTAP* gene. Beyond the age of 6 months, typical manifestations become apparent with coarse facies, skeletal disproportion, and organomegally.

Homozygous TRPV6 mutations present with clinical, radiological and biochemical features identical to i-cell disease (**Figures 1-5a,b**). TRPV6 (the transient receptor potential cation channel, subfamily V, member 6) plays an important role in materno-fetal calcium transfer. Short long bones, bowed femora, rib deformities and Intrauterine Growth Restriction (IUGR) are reported antenatally (35). After birth the bone disease rapidly heals with enteral calcium supply available for mineralization. Radiological signs completely resolve by 18 months to 2 years of age.

Miscellaneous Bone Conditions Presenting in the Neonatal Period

Transient neonatal hyperparathyroidism and bone disease similar to above situations, has been described in maternal hypoparathyroidism (36). In some cases, previously unknown maternal disease may be diagnosed after the birth of an affected baby. Osteopetrosis can present in the neonatal period with hypocalcaemia and high PTH with dense, osteosclerotic bones on X-ray. Osteopenia of prematurity is another condition that may manifest with pathologic rib and long bone fractures. As most materno-fetal calcium transfer during pregnancy occurs during the last trimester, babies born <28 weeks gestational age are particularly predisposed to this condition. A comprehensive review of the diagnosis and management of this condition has been published recently (37).

INVESTIGATIONS

With advancement in technology and expertise, 3D antenatal ultrasonography and fetal MRI as an adjunct when spinal abnormalities are suspected, are increasingly used in prenatal diagnosis of potential bone disorders (38). Good quality antenatal imaging influences further targeted molecular genetic testing, invasive prenatal diagnosis in at risk families, antenatal counseling, informing obstetricians of the best mode of delivery and perinatal management.

Postnatal x-rays, with or without a complete skeletal survey, are the most useful investigation in diagnosing both structural (with normal bone biochemistry) and bone mineralization defects (with abnormal bone biochemistry) in the neonatal period. Radiological features suggesting hyperparathyroidism viz undermineralization, periosteal cloaking, metaphyseal changes, and subperiosteal resorption (diaphyseal tunneling) along with an elevated PTH, suggest primary or secondary hyperparathyroidism (**Figure 2**).

First line biochemical investigations include bone profile (including serum calcium, phosphate, and alkaline phosphatase levels), parathyroid hormone and 25 hydroxy vitamin D levels. When calcium levels are only marginally elevated, consider further investigations including maternal calcium and PTH profile, baby's urine for glycosaminoglycans and enzyme assay (for i-cell disease). Based on clinical, radiological, and biochemical abnormalities, targeted molecular genetics such as Type 1 collagen mutation, ALPL, FGFR3 CaSR, TRPV6, and GNPTAB, to name a few should be considered to confirm the diagnosis. In a few cases, despite extensive genetic investigations, the cause for the bone disorder may remain unknown.

Children with complex neonatal bone disorders should ideally be managed in a tertiary pediatric unit by a multidisciplinary team comprising of a pediatric endocrinologist, geneticist, radiologist, orthopedic and neurosurgeon, dentist, physiotherapist and occupational therapist, clinical psychologist, specialist bone nurses, and social worker to provide the necessary family support.

CONCLUSION

Neonatal bone health is of growing interest not only due to the impact initial management can have on bone health during childhood, adolescence, and early adulthood but also the need for early and accurate diagnosis and initiation of life saving treatment such as enzyme replacement therapy in conditions such as hypophosphatasia. Neonatal bone disorders are a rapidly developing area of research interest with interventional studies and drug trials targeting bone health as early as the antenatal period in conditions such as osteogenesis imperfecta or in the neonatal period for Achondroplasia. However, the mainstay of managing neonatal bone disorders remains follow up by a specialist multidisciplinary team to achieve best possible functional outcomes.

AUTHOR CONTRIBUTIONS

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

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REVIEW
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Disorders/Differences of Sex Development Presenting in the Newborn With 46,XY Karyotype

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Differences/disorders of sex development (DSD) are a heterogeneous group of congenital conditions, resulting in discordance between an individual's sex chromosomes, gonads, and/or anatomic sex. The management of a newborn with suspected 46,XY DSD remains challenging. Newborns with 46,XY DSD may present with several phenotypes ranging from babies with atypical genitalia or girls with inguinal herniae to boys with micropenis and cryptorchidism. A mismatch between prenatal karyotype and female phenotype is an increasing reason for presentation. Gender assignment should be avoided prior to expert evaluation and possibly until molecular diagnosis. The classic diagnostic approach is time and cost-consuming. Today, a different approach may be considered. The first line of investigations must exclude rare life-threatening diseases related to salt wasting crises. Then, the new genetic tests should be performed, yielding increased diagnostic performance. Focused imaging or endocrine studies should be performed on the basis of genetic results in order to reduce repeated and invasive investigations for a small baby. The challenge for health professionals will lie in integrating specific genetic information with better defined clinical and endocrine phenotypes and in terms of long-term evolution. Such advances will permit optimization of counseling of parents and sex assignment. In this regard, society has significantly changed its attitude to the acceptance and expansion beyond strict binary male and female sexes, at least in some countries or cultures. These management advances should result in better personalized care and better long-term quality of life of babies born with 46,XY DSD.

Keywords: 46,XY disorder of sex development, testis, fetal gonadal hormones, ambigous genitalia, sex assignment

Si sta come
d'autunno
sugli alberi
le foglie
G. Ungaretti (1918)

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INTRODUCTION

Phenotypic sex is the result of a coordinated and sequential series of fetal events controlled by complex gene systems, transcription factors, and optimal hormone secretion during critical developmental windows (1–4). Sex development starts at fertilization by the establishment of chromosomal sex (XX or XY). In human fetuses with XY karyotype, the *SRY* (*sex determining region on the Y chromosome*) and the related gene network promote the formation of functional testes (sex determination). The final step (sex differentiation) leads to the formation of the phenotypic sex (i.e., development and stabilization of the external and internal genitalia as well as the programming of the male or female brain and reproductive axis). In the 46,XY fetus, this step is based on the hormones secreted by primordial testes and peripheral response of target tissues to these hormones (1–4) (**Figure 1**).

Disorders (or differences) of sex development (DSD) are defined as congenital conditions which feature an alteration in the development of genetic, gonadal, or phenotypic sex (5, 6). This terminology recognizes the simple but fundamental pathway of the nature of sex chromosomes (XX or XY) organizing the development of the gonads (testis or ovary) whose hormones (effectively anti-Müllerian hormone and androgens in fetal life) determine the genital phenotype (male or female) (7). The 46,XY DSD group includes a wide spectrum of conditions due to genetic variants, altered hormonal secretion, or abnormal peripheral sensitivity to testicular hormones that are able to change the usual male fetal development, causing varying degrees of under-virilization (5–7). 46,XY DSD may be divided into two broad categories: (1) disorders of sex determination characterized by abnormal gonadal development; (2) disorders of sex differentiation characterized by altered production of testicular hormones or altered peripheral response to steroid or protein hormones produced by the fetal testis (**Table 1**).

The impact of 46,XY DSD in the life of the affected individuals and their families is immense, as these conditions require long-term clinical, endocrinological, and psychological management (5). Adequate management of the newborn with 46,XY DSD is challenging, because it affects sex assignment (and possible re-assignment), decisions on gonadal management (including oncological risk), hormone replacement therapy from adolescence onward (when needed), and lifelong health status (3, 5–8). Early correct diagnosis is a key factor for optimizing quality of life, but true diagnoses based on pathogenetic pathways is still not reached in some individuals (9–14), jeopardizing outcome.

In this paper, some aspects related to the diagnosis and management of newborns with 46,XY DSD are discussed, taking into consideration some personal views developed during years of clinical work and exchange of opinions with our colleague and friend Paolo Ghirri, a frontier soldier in the field of neonatal endocrinology.

CLINICAL PRESENTATION

Presentation of a newborn with 46,XY DSD may be characterized by varying degrees of ambiguity of genital phenotype, usually leading to easy identification during routine physical examination. In some instances, few clinical signs, such as mono- or bilateral inguinal herniae or mild hypospadias or micropenis associated with undescended testes, may be the only manifestations (4–6) (**Table 2**). Accurate phenotypic examination (appearance of the external genitalia, presence or absence of palpable gonads, measurement of the phallus or clitoral length, identification of the position of the urethral opening, presence or absence of a vagina or urogenital sinus) must be made (4, 6, 8, 15). A complete female phenotype or very mild undervirilization may delay the diagnosis for

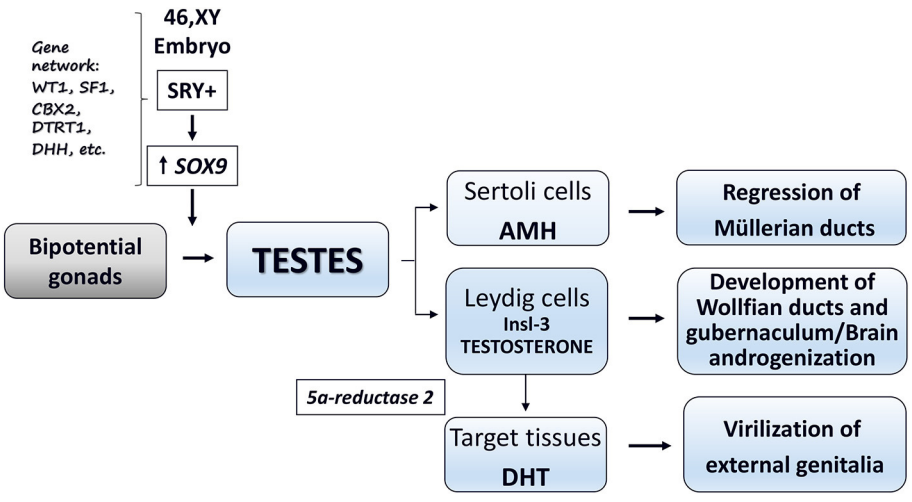

FIGURE 1 | Schematic representation of male sex differentiation *in utero*.

TABLE 1 | Main forms of 46,XY DSD (4, mod).

Disorders of gonadal (testis) development
- Complete or partial gonadal dysgenesis (due to genetic variants in SRY, SOX9, NR5A1, WT1, DHH, DMRT1, etc.)
- Ovotesticular DSD
- Testis regression
Disorders of androgen synthesis
- LH receptor mutations
- Smith-Lemli-Opitz syndrome
- Steroidogenic acute regulatory protein mutations*
- Cholesterol side-chain cleavage (CYP1IA1)*
- 3β-hydroxysteroid dehydrogenase 2 (HSD3B2)*
- 17α-hydroxylase/17,20-lyase (CYP17)*
- P450 oxidoreductase (POR)
- 17β-hydroxysteroid dehydrogenase (HSD17B3)
- 5α-reductase2 (SRD5A2)
Disorders of androgen action
- Androgen insensitivity syndrome (complete, partial, minimal)
- Drugs and environmental modulators of androgen receptor activity
Disorders of AMH synthesis or action
- Persistent Müllerian duct syndrome
Other
- Syndromic associations of male genital development (e.g. cloacal anomalies, Robinow, Aarskog, Hand-Foot-Genital, syndromes)
- Vanishing testis syndrome
- Isolated hypospadias (CXorf6)
- Congenital hypogonadotropic hypogonadism
- Cryptorchidism (INSL3, GREAT)
- Environmental endocrine disruptors

**Associated with congenital adrenal hyperplasia.*

months or years [as may occur in complete and minimal androgen insensitivity syndrome (AIS) or complete gonadal dysgenesis]. Salt-losing crises due to adrenal insufficiency rarely occur in 46,XY DSD (**Table 1**) (5, 6, 8, 15). Valuable clinical scores were developed to grade the atypical genitalia (16, 17). Some well-written reviews or guidelines are available on how to perform the physical evaluation of neonatal genitalia (5, 6, 8, 18, 19). Readers are encouraged to refer to these for a detailed description, but neonatal phenotypes may be inconclusive for diagnosis in the absence of a clear family history (**Table 2**).

Prenatal diagnosis may occur due to the appearance of atypical genitalia on prenatal ultrasound or a mismatch between phenotype and genotype or a suggestive family history (20, 21). The growing use of prenatal genetic tests and high-resolution ultrasound is likely to increase the detection of fetuses with genotype/phenotype sex mismatch during pregnancy (22). The management of these conditions is a new challenge that requires expert counseling. Some genetic investigations could be performed prenatally, when possible. Complete evaluation should be performed after delivery to reach a correct diagnosis and to program personalized management. Prenatal diagnosis permits the opportunity for counseling and education of parents prior to the birth of a child with 46,XY DSD (8).

DIAGNOSTIC PROCEDURES

Rational investigations are mandatory in a newborn with 46,XY DSD to avoid repeated and invasive tests in a small baby (22). Balsamo et al. (19) proposed an extensive diagnostic scheme of laboratory assessment in the first 24–48 h of life (**Figure 2**, left panel). Such a scheme is still appropriate to avoid a salt-losing crisis, caused by rare forms of adrenal insufficiency (**Table 1**). During minipuberty (15–90 days after birth), hormonal status should be re-evaluated (8). This scheme is time and cost consuming and it may not result in a specific diagnosis because of the difficulties in steroid determination and because testicular protein hormone assays are unavailable in some clinical laboratories and countries (8, 23). Therefore, a parallel approach should be considered (24) (**Figure 2**, right panel). This approach suggests the use of advanced genetic technologies (i.e., next generation sequencing, whole exome sequencing, targeted CGH array) as the first-line test after karyotyping (24) which may result in a molecular diagnosis. After a genetic diagnosis, selected investigations should be performed to detail the clinical and biochemical phenotype, minimizing unnecessary tests, sampling, and analyses. The molecular diagnosis will permit more rational sex assignment, recognizing the natural history of the identified 46,XY DSD (8, 18), the risk of gonadal neoplasia (25), the possibility for fertility (26, 27), and mental health (8). In addition, this approach may aid the understanding of the clinical and molecular characteristics of emerging DSD associated with oligogenic mutations, in which multiple hits may contribute to the phenotype (28). In our experience, patients presenting between 2007 and 2016 had a higher rate of correct diagnosis and reduced diagnostic delay in comparison with those presenting between 2000 and 2006. The advent of new genetic techniques strongly influenced this result (14).

A recent position paper not specific for DSD from European Reference Network on rare endocrine conditions (ENDO-ERN, www.endo-ern.eu) concluded that early diagnosis of a genetically based endocrine disorder contributes to precise management and helps the patients and their families in their self-determined planning of life (29). Furthermore, the identification of a causative genetic alteration allows an accurate prognosis of recurrence risks for family planning. Asymptomatic carriers of pathogenic variants can be identified, and prenatal testing might be offered, where appropriate (29). Pitfalls leading to potentially inconclusive results may be due to identification of variants of unknown significance and inconsistent associations between DSD phenotypes and molecular findings (8). Costs and availability of the new genetic technologies may be additional factors limiting their application in some clinical settings (8), which might be overcome by establishing centers of expertise at national levels or by international consortia.

SEX ASSIGNMENT

Sex assignment is one of the main issues in the management of a newborn with 46,XY DSD (8, 19).

In the past, the "optimal gender policy" hypothesis stated that gender identity was neutral at birth and developed in the

TABLE 2 | Clinical findings of main 46,XY DSD (without adrenal insufficiency).

	46,XY gonadal dysgenesis	NR5A1 deficiency*	Leydig cell hypoplasia	17β-HSD3 deficiency	5α-reductase 2 deficiency	Complete/partial/ minimal Androgen resistance
Prevalence	?	?	Very rare	1: 147.000°	? °	1: 20/90.000
Inheritance	Variable	AD	AR	AR	AR	X-linked
Gene	*SRY, DHH,* etc.	*NR5A1*	*LHR*	*17β-HSD3*	*SRD5A2*	*AR*
Chromosome	variable	9q33.3	2p21	9q22	2p23	Xq11–12
External genitalia	Female	Female to male	Female to ambiguous	Female to ambiguous	Female to ambiguous	Female to ambiguous to male
Wolffian structures	No	Variable	No	variable	Yes	No (complete) to variable to male (minimal)
Müllerian structures	Yes	Variable	No	No	No	No
Gonads	Streak	Testes	Testes	Testes	Testes	Testes
Puberty	No	No/virilization	No	Virilization	Virilization	Femminilizatio to virilization
Sex change	No	Sometimes	No	30–50%	~75%	No (complete/minimal) to sometimes (partial)

AD, autosomal dominant; AR, autosomal recessive.
**No adrenal insufficiency in heterozygous state; adrenal insufficiency is operative in homozygous state.*
°Frequent in some specific populations with a high rate of consanguineous marriage.

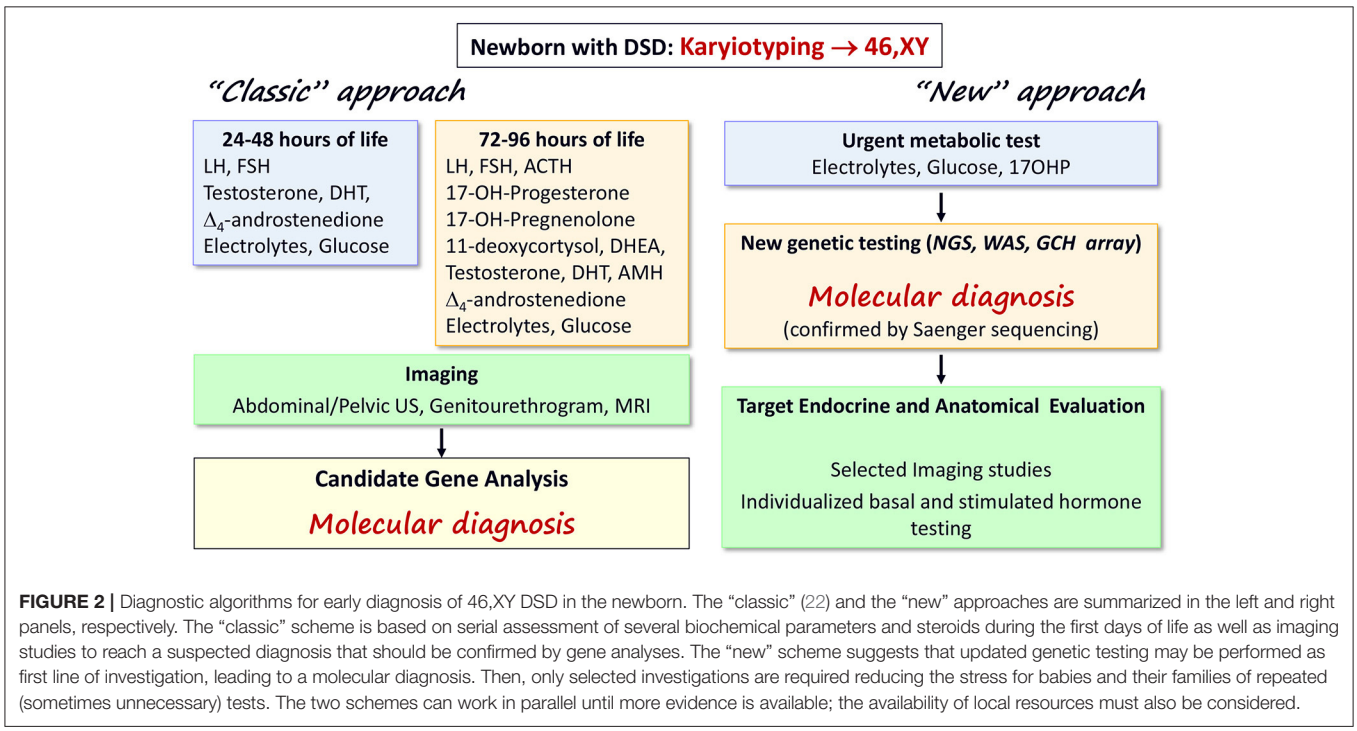

FIGURE 2 | Diagnostic algorithms for early diagnosis of 46,XY DSD in the newborn. The "classic" (22) and the "new" approaches are summarized in the left and right panels, respectively. The "classic" scheme is based on serial assessment of several biochemical parameters and steroids during the first days of life as well as imaging studies to reach a suspected diagnosis that should be confirmed by gene analyses. The "new" scheme suggests that updated genetic testing may be performed as first line of investigation, leading to a molecular diagnosis. Then, only selected investigations are required reducing the stress for babies and their families of repeated (sometimes unnecessary) tests. The two schemes can work in parallel until more evidence is available; the availability of local resources must also be considered.

postnatal period under the influence of social, familial, and cultural factors (30). According to this theory, if a child with a DSD is raised without gender and anatomical sex ambiguity, gender identity was expected to develop in line with assigned sex (30). This hypothesis determined the practice of early sex assignment; early genital surgery was consequently performed "to correct" the atypical genitalia according to assigned sex. Long-term studies showed that the "optimal gender policy" did not always lead to a satisfying adult quality of life and sexuality (31). In recent years, the management of 46,XY DSD has changed. New ideas on psycho-sexual development as the result of multifaceted genetic, hormonal, and psychosocial influences have arisen (3, 32–36). Both biological sex and psychosexual development are considered as a spectrum of

TABLE 3 | 46,XY DSD: similar phenotypes, different decisions, different outcome in two people with 46,XY karyotype and atypical genitalia at birth.

Person	Mary	Mario
Description of phenotype at birth	Ambiguous genitalia with clitoromegaly, urogenital sinus, inguinal gonads	Ambiguous genitalia with severe proximal hypospadias, urogenital sinus, inguinal gonads
Assigned sex	Female	Male
Investigations	Repeated endocrine and imaging studies	Imaging study of genitalia
Early diagnosis	Gonadal dysgenesis in infant with Morris syndrome*	Male undervirilization
Procedures	Gonadal removal, feminizing surgery	Male reconstructive surgery
Adult outcome	Gender dysphoria ("I'm sick, I can't understand what I am") plus other psychiatric disorders, social withdrawal, poor education level, and work opportunity	Married, satisfying social and sexual activity, spontaneous proven fertility (2 daughters), University degree, top positions in his work
Age at molecular diagnosis	30 years old	66 years old
Molecular diagnosis	Compound heterozygosity for *SRD5A2* gene variants	Compound heterozygosity for *SRD5A2* gene variants

The same molecular diagnosis (compound heterozygosity for SRD5A2 gene variants) was made in adulthood.

**Complete androgen insensitivity does not present with ambiguous genitalia and functioning testes are present and therefore not "gonadal dysgenesis".*

possibilities rather than a simple binary male/female system (37, 38).

A more open approach is needed in babies where the sex may not be easily defined at birth and more time is needed in order to determine the natural inclination of an individual partly related to their prenatal hormonal milieu (33). For example, 46,XY individuals with *SRD5A2* deficiency assigned as female at birth showed high rates of sex change and gender dysphoria (56–63%) from adolescence onward (39, 40). Functional brain imaging studies of women with complete AIS in comparison with 46,XY male and 46,XX female controls suggest that testosterone modulates the microstructure of somatosensory and visual cortices and their axonal connections to the frontal cortex; testosterone may also influence functional connections from the amygdala (35). The high rate of gender role switch from female to male in some 46,XY DSD during puberty may be due to the prenatal brain androgenization from normal testosterone secretion during intrauterine life (41) (**Table 2**). In these individuals, different decisions at birth can determine different outcomes in adulthood (**Table 3**).

Although there is still an association between the external appearance of the genitalia and the choice of sex assignment, clear temporal trends pointing toward an increased likelihood of infants with 46, XY DSD being raised as boys has been reported (42). Some factors may explain the new tendency for male gender assignment in some 46,XY DSDs. Fertility potential has become an important issue to evaluate, because spontaneous or assisted paternity has been documented in some men with 46,XY DSD (26). Furthermore, new data showed that the gonadal cancer risk is relatively low in several forms (18, 25, 43). Thus, recommending early gonadectomy may not be necessary, since regular follow-up could be an adequate approach (41, 43). In addition, new RNA microarray technology is likely to lead to very early identification of gonadal neoplasia (44, 45). The past recommendation for female assignment based on easier surgery has been overcome by improvements in male reconstructive surgical techniques. However, some studies have reported that the majority of individuals with 46,XY DSD raised as females have not experienced gender dysphoria (46, 47). Thus, male gender assignment should not be the rule in every case.

Social and cultural factors may influence decisions on sex assignment and outcome (48). In some societies, female infertility precludes marriage, which also affects employment prospects and creates economic dependence. Religious and philosophical views may influence how parents respond to the birth of an infant affected by 46,XY DSD. There may be fatalism and guilt feelings related to congenital malformations or genetic conditions; poverty and illiteracy may impair access to health care or may preclude the availability of updated knowledge and new technologies (18, 19, 47, 48).

Because the long-term outcome of the early management of babies with 46,XY DSD remains largely based on evidence from small series or single reports, ethical guidelines for the management of infants with DSD must be taken into consideration (49). These state that the following principles should guide clinical decisions: minimizing physical and psychosocial risks, preserving the potential for fertility and satisfying sexual relations in adolescence and adulthood, leaving options open for the future if necessary, respecting the parents' wishes, beliefs and sociocultural tradition, when possible, to guarantee the best options for a healthy life (that is *a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity; WHO, 1948*) (49). Future studies integrating genetic, endocrine, imaging, surgical, psychologic, and follow-up data will give more objective data to aid sex assignment.

MULTIDISCIPLINARY TEAMS

Each subject with 46,XY DSD should receive individualized care by an expert multidisciplinary team. This team should include medical specialists (pediatric endocrinologists, geneticists, reproductive medicine specialists, pediatric surgeons and urologists, mental health specialists, ethicists, etc.) as well as nurses, social workers, and patient associations to optimize family-centered care (5, 8, 15, 18). The teams should be available at reference centers clearly delineated in each country and they should work closely with smaller centers (hub and spoke model), because the birth of a baby with 46,XY DSD can occur in any neonatal unit. The multidisciplinary teams should collaborate in communicating the correct information on DSD to the parents as well as pros and cons of management. The multidisciplinary

teams should help and support the anxieties of parents that may lead to premature and irreversible decisions (47). They should also share advanced knowledge (including by e-learning projects), diagnostic procedures, and facilities for patients. The team should include the laboratories performing analyses for DSD (19, 23) and should operate within a quality framework and actively engage in harmonization of diagnostic and management approaches, permitting sound comparable data. The DSD–Endo-ERN as well as the international Disorders of Sex Development (I-DSD) and International Congenital Adrenal Hyperplasia registry (I-CAH) registries are relevant examples of tools for improving practice by virtual expert networks, cooperation between expert healthcare centers, and multicenter research on rare disorders (50).

SUPPORT GROUPS

Support groups may be invaluable to individuals with 46,XY DSD and their families (51, 52). They actively work to improve management and research in this field and to push healthcare systems toward higher standards of care (51). For couples expecting a baby with a genetic/phenotypic sex mismatch or parents of a newborn with 46,XY DSD, support groups may provide a context in which intimate issues of concern can be approached by sharing parents' and patients' experiences. Support groups can also help families find the best quality of care (*www.dsd.guidelines.org*; *www.dsd-life.eu*) (51, 52). Parents of a baby with 46,XY DSD should be encouraged to contact a dedicated support group to share emotions and information.

Concerns have been expressed about the authority of LGBT (lesbian, gay, bisexual, and trans) movements in representing the community of people with DSD, which might lead to misleading messages related to confusion in terminology or the clinical condition (53).

CONCLUSIONS

The care of people with DSD quickly evolves as knowledge in this field accrues (4–8), but the birth of a baby with 46,XY DSD is still perceived as a "social" rather than a true "medical" emergency. Currently, there are no fully established or evidenced based "right" or "wrong" decisions in this difficult field, but every family has to find its own path with open support and objective information from expert teams when dealing with the specific nature of their child (27, 33, 54). While many patients fare well and have a good quality of life (46, 47, 55), other individuals have expressed uncertainty about belonging to a specific gender or have reported poor quality of life (38, 56) (**Table 3**). New knowledge, updated investigations, clear diagnoses, respect for newborns and their families, and improved collaboration among national and international networks are likely to result in better health of people with DSD. Support groups provide added value to help families and promote quality research and care. Qualified psycho-social care should be also planned to optimize lifelong quality of life. Improvements are needed on diagnostic schemes in the first days of life as well as objective criteria to assign sex, to predict the risk of germ cell cancers and unnecessary gonadal removal, and to optimize surgical procedures and future fertility options. We outline a different approach to the investigation of 46,XY DSD which involves genetic testing, following exclusion of a salt-losing crisis, as genetic testing advances and becomes more available in this area.

AUTHOR CONTRIBUTIONS

All authors conceived the paper, have written the first draft, revised, and approved the final paper.

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SYSTEMATIC REVIEW
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Analysis of the Screening Results for Congenital Adrenal Hyperplasia Involving 7.85 Million Newborns in China: A Systematic Review and Meta-Analysis

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Background: Congenital adrenal hyperplasia (CAH) is a group of congenital genetic diseases caused by defective steroidogenesis. Our study aims to systematically analyze the screening results for CAH in Chinese newborns.

Methods: Studies were searched from PubMed, Web of Science, Cochrane library and some Chinese databases up to September, 2020. Meta-analysis was performed after quality assessment and data extraction.

Results: After a review of 2 694 articles, we included 41 studies enrolling 7 853 756 newborns. In our study, we found that the incidence of CAH in China was 0.43‰ [95% confidence intervals(CI), (0.39‰, 0.48‰)], or 1/23 024 [95%CI, (1/25 757,1/20 815)]. 27 studies were included for analysis of the screening positive rate, which gave a rate of 0.66% [95%CI, (0.54%, 0.78%)]. As for the recall rate of positive cases, 17 studies were included and showed that the recall rate reached 86.17% [95%CI, (82.70%, 89.64%)]. Among the CAH patients, the ratio of males to females was 1.92:1 (119:62), and the ratio of salt wasting (SW) to simple virilization (SV) type was 3.25:1 (104:32). The average 17-hydroxyprogesterone (17-OHP) value of CAH was 393.40 ± 291.85 nmol/L (Range 33-1 300 nmol/L); there was no significant difference between male and female patients (437.17 ± 297.27 nmol/L v.s. 322.25 ± 293.04 nmol/L, P=0.16), but a significant difference was found between SW and SV patients (483.29 ± 330.07 nmol/L v.s. 73.80 ± 7.83nmol/L, P=0.04).

Conclusion: We systematically analyzed the current situation of neonatal CAH screening in China, which will deepen our understanding for future CAH screening and early diagnosis.

Keywords: neonatal screening, incidence, congenital adrenal hyperplasia, 17-OHP, meta-analysis

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INTRODUCTION

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive inherited diseases caused by defects of essential enzymes in the synthesis of steroid hormones. Because of different degrees of aldosterone and cortisol deficiency, classical CAH mainly manifests with salt-wasting symptoms and SV type mainly with hyperandrogenism. Many studies have shown that CAH patients often have some

adverse outcomes during childhood or adulthood (1, 2). Therefore, early screening, early diagnosis and early treatment are particularly critical to help patients with CAH to have normal and healthy development.

About 90-95% of CAH cases are caused by deficiency of steroid 21-hydroxylase (21-OHD), characterized by elevated 17-hydroxyprogesterone (17-OHP) and reduced glucocorticoid levels. The current screening for CAH is still dominated by 21-OHD, although some rare types such as 11β-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiency may also be found. Screening for CAH was first performed in United States of America in 1977, and currently more than 35 countries have carried out CAH screening (1, 2). In China, such a screening program started in the early 1990s, and to date, many screening centers have obtained regional incidence data. However, due to China's vast territory and unbalanced medical provision, CAH screening coverage rate in China was only 18.9%-19.9% according to the statistics of newborn screening in 2013 (3). In addition, there were significant differences in reports of the incidence of CAH, for example, the 2016 CAH guideline (China) stated that the domestic incidence was 1/16 466-1/12 200 (1), while the 2018 Endocrine Society CAH guideline stated that the incidence of CAH in China was as high as 1/6 064 (sample size 30 000 cases) (2). National newborn screening is the only way of obtaining precise incidence data of CAH in China and promote its early diagnosis, but currently there are still many difficulties in carrying out such a national screening program.

Therefore, we used the method of meta-analysis to comprehensively analyze the results of CAH newborn screening in different regions of China, and conducted a systematic analysis of its screening positive rate, recall rate and incidence of CAH. Our study will help us understand the screening status and promote an effective CAH neonatal screening program in the future.

METHODS

Data Sources and Searches

We developed a protocol for the meta-analysis and followed the principles of the PRISMA statement (see **Supplementary Table 1**). Relevant studies were searched from PubMed, Web of Science, Cochrane library and some Chinese databases (CNKI, Wanfang, VIP and CBMD) up to September, 2020. Our searches were based on combinations of the following index terms: newborn screening, congenital adrenal hyperplasia, CAH, 17-hydroxyprogesterone, 17-OHP or 17α-OHP and the corresponding terms in Chinese. We also reviewed the reference lists of retrieved studies and review articles.

Eligibility Criteria and Exclusion Criteria

The studies would be included if they met following criteria: (1) Results of CAH newborn screening in different provinces, cities and autonomous regions of China; (2) Sample collection was subject to "Technical Specifications for Blood Collection for Neonatal Disease Screening(China)" or the regional handbook (72 hours after the birth, blood is collected from the inside or outside of the heel to form dried blood spots, which are naturally dried and stored in a refrigerator at 2-8°C, and then sent for testing); (3) Detection methods: dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA) or enzyme-linked immunosorbent assay (ELISA) was used to quantitatively measure 17-OHP values of dried blood spots (Most Chinese laboratories recognize 30 nmol/L as the positive cut-off value, only the Children's Hospital of Shanghai Jiaotong University takes 40 nmol/L as the cut-off value); (4) The main indicators are the incidence of CAH, the positive rate, the recall rate and some other characteristics related to CAH.

The following exclusion criteria were applied: (1) Studies with overlapping screening regions or screening time; (2) Not meeting the requirements of the eligibility criteria; (3) Studies with low quality. In addition, studies which were not published in English or Chinese were also excluded because of language limitations.

Data Collection and Quality Assessment

According to the above eligibility criteria and exclusion criteria, a data extraction table was developed and relevant data were collected. The information included: authors, published year, screening year and participants, positive cases and positive rate, recall cases and recall rate, diagnosed cases and their characteristics (gender, clinical classification and 17-OHP levels), etc.

An 11-item checklist recommended by the Agency for Healthcare Research and Quality of America (AHRQ) (see **Supplementary Table 2**) was used to evaluate the quality of included studies. An item would score "0" with answer "NO" or "UNCLEAR"; otherwise, it would score "1". With a total score of 11 points, article quality was assessed as follows: low quality = 0-3, moderate quality = 4-7, high quality = 8-11. Two reviewers individually assessed the quality of eligible studies, and a senior investigator resolved the discrepancies if necessary.

Summary Measures and Synthesis of Results

We used the Stata 12.0 software to analyze the data. If different units were used in the studies, they were converted to international standard units. The effect size in our study was shown as "rate" and its 95% confidence interval (95% CI). I^2 and Chi^2 tests were used to estimate the heterogeneity, with I^2 value less than 50%, heterogeneity was considered to be small and a fixed effect model was used; otherwise, the random effect model was used. Subgroup analysis was also conducted to identify the possible sources of heterogeneity. Publication bias was shown by a funnel plot and evaluated by the Begg's test. Independent sample t test was used for statistical analysis, $P<0.05$ indicated that the difference was statistically significant.

RESULTS

Study Selection

Our initial data search yielded a total of 2 694 articles (1 747 articles in Chinese and 947 in English). 2 352 articles were

Abbreviations: CAH, congenital adrenal hyperplasia; SW, salt wasting; SV, simple virilization; 17-OHP, 17-hydroxyprogesterone; 21-OHD, 21-hydroxylase deficiency; DELFIA, dissociation-enhanced lanthanide fluorescence immunoassay; ELISA, enzyme-linked immunosorbent assay; LC-MS/MS, liquid chromatography-tandem mass spectrometry; AHRQ, Agency for Healthcare Research and Quality of America; CI, confidence interval.

excluded by reading the titles and abstracts, and 266 were excluded because they didn't meet the eligibility criteria, whereas the remaining 76 were considered as potentially eligible for our analysis. After careful reading of the entire full text, 41 articles with moderate or high quality met the eligibility criteria and were included in the meta-analysis. A flow diagram (**Figure 1**) shows the flow chart of the literature search.

Quality Assessments

In our included studies, the collection of specimens abided by the "Technical Specifications for Blood Collection for Neonatal Disease Screening" or the guidelines of the corresponding region; the DELFIA or ELISA method was used to detect the 17-OHP concentration of dried blood spot specimens; the main indictors were incidence rate, the positive rate and recall rate of screening, etc.

Based on AHRQ quality assessment items, 41 studies (4–44) that scored four or more were deemed as moderate or high quality. The average score of 7.7 indicated minimal risk of bias. The results are shown in **Table 1** and **Supplementary Table 2**.

Study Characteristics

After quality assessments, 41 studies (4–44) with 7 853 756 newborns were included, and 381 cases were diagnosed with

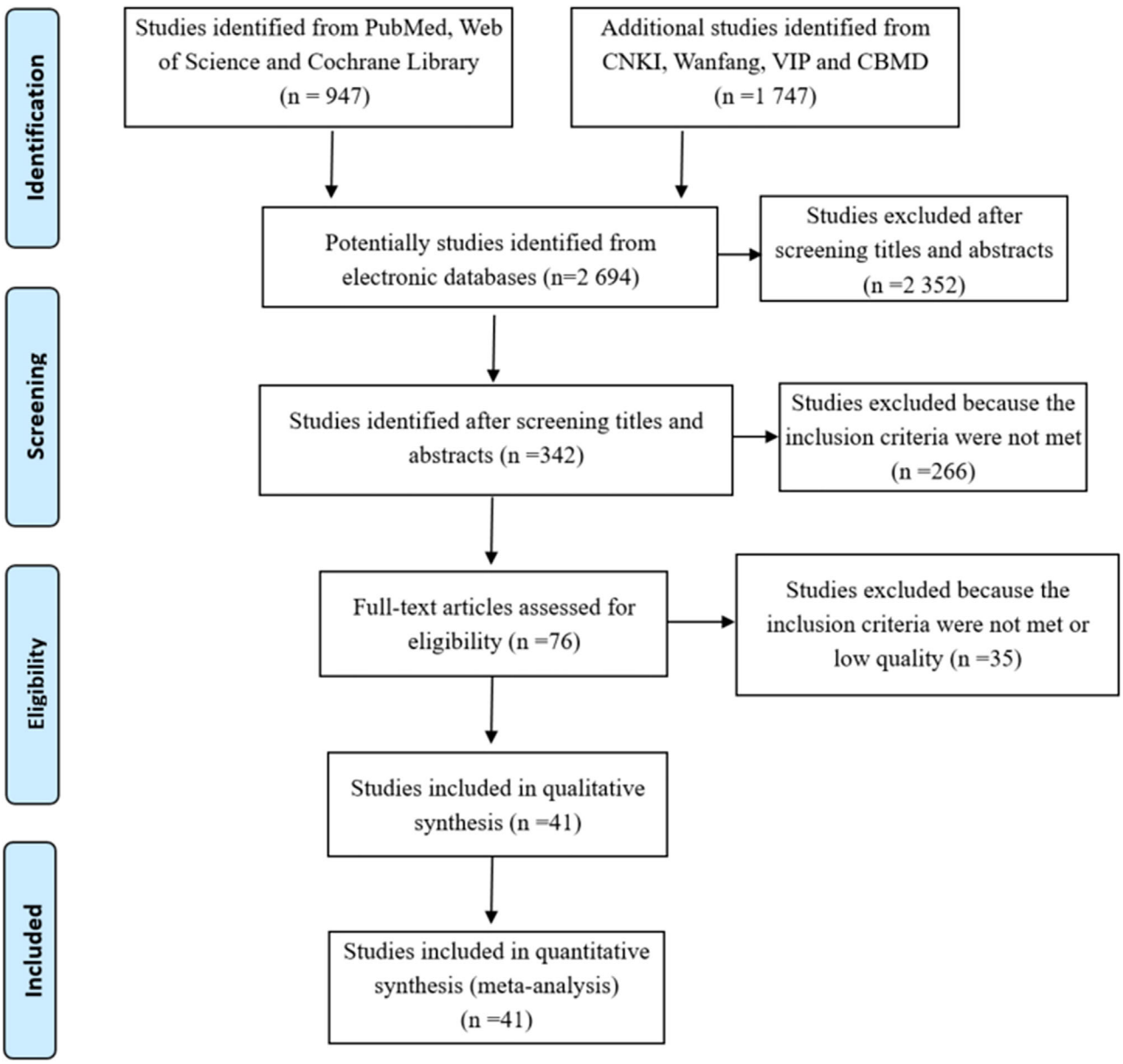

FIGURE 1 | Flowchart depicting literature search and selection (Follow the PRISMA Flow Diagram, for more information, visit www.prisma-statement.org).

TABLE 1 | Characteristics of studies included in the meta-analysis.

Area		Years	Screening Cases	CAH cases	Incidence	Positive cases (rate %)	Recalled cases (rate %)	Male : Female	SW : SV	AHRQ scores
Province	**City**									
Taiwan (4)		2000-2001	192 687	13	1/14 822			6: 7	9: 4	8
Shanghai (5)		2007-2008	93 971	5	1/18 794	214(0.23)	176(82.24)	4: 1		9
Hunan (6)		2009-2013	40 988	4	1/10 247	1 192(2.91)	1 120(93.96)	2: 2	1: 3	9
Guangxi (7)		2012-2015	378 252	22	1/17 193	1682(0.44)				7
Ningxia (8)		2014-2016	160 046	11	1/14 550	70(0.04)	70(100)	6: 5	9: 2	9
Beijing (9)		2014-2017	22 632	2	1/11 316	156(0.69)		2: 0		7
Sichuan (10)		2015-2018	271 283	16	1/16 955				14: 2	9
Shanxi (11)		2015-2016	64 378	3	1/21 459	323(0.50)	297(91.95)	2: 1		9
Zhejiang	Ningbo (12)	2014	88 406	3	1/29 469	517(0.58)		2: 1	3: 0	8
	Others (13)	2014-2016	1 719 510	69	1/24 920					6
Shandong	Jinan (14)	2003-2011	88 350	11	1/8 032			10: 1	9: 2	8
	Taian (15)	2010-2012	161 337	8	1/20 167	1 401(0.87)	1 386(98.93)			8
	Liaocheng (16)	2009-2010	76 383	5	1/15 277	1 456(1.91)	1 235(84.82)			5
	Linyi (17)	2009-2013	740 730	24	1/30 864			12: 12		7
	Heze (18)	2013	119 560	3	1/39 853					5
	Zibo (19)	2010-2014	178 577	11	1/16 234	2 875(1.61)	2 687(93.46)	7: 4	7: 4	9
	Weifang (20)	2012-2015	305 879	14	1/21 849	3 448(1.13)	3 354(97.27)	11: 3	11: 3	8
	Rizhao (21)	2012-2014	101 161	9	1/11 240					5
	Qingdao (22)	2013-2017	566 395	32	1/17 700	2 536(0.45)	2 310(91.09)	22: 10		9
Guangdong	Zhongshan (23)	2008-2010	105 320	2	1/52 660	307(0.29)	168(54.72)	2: 0		9
	Foshan (24)	2010-2011	74 791	5	1/14 958	260(0.35)			2: 3	9
	Shenzhen (25)	2010-2011	329 135	15	1/21 942	1 581(0.48)	1 113(70.40)		13: 2	9
	Dongguan (26)	2009-2013	551 538	17	1/32 443	2 757(0.50)	2 453(88.97)	11: 6	11: 6	9
	Heyuan (27)	2014-2016	45 000	4	1/11 250					7
Jiangsu	Nanjing (28)	1993-2002	103 935	5	1/20 787	401(0.39)		3: 2		8
	Wuxi (29)	1992-2006	61 284	4	1/15 321			3: 1		8
	Changzhou (30)	2001-2010	175 876	13	1/13 529					8
	Suzhou (31)	2010-2012	96 423	5	1/19 285	864(0.90)	464(53.70)	4: 1	4: 1	8
	Yancheng (32)	2012-2014	199 612	9	1/22 179	366(0.18)				9
	Lianyungang (33)	2016	53 305	3	1/17 768	265(0.50)	265 (100)	2: 1	3: 0	9
	Yangzhou (34)	2013-2017	88 829	4	1/22 207	240(0.27)	238(99.17)	3: 1	4: 0	9
Jiangxi	Nanchang (35)	2011-2013	27 988	2	1/13 994	448(1.60)	379(84.60)	2: 0	2: 0	9
	Jiujiang (36)	2015-2017	25 000	3	1/8 333	29(0.12)				7
	Yichun (37)	2016-2017	80 305	4	1/20 076	132(0.16)	112(84.85)	3: 1		9
Chongqing	Yuzhong (38)	2012-2017	125 320	7	1/17 903					5
	Others (39)	2012-2017	25 958	1	1/25 958	21(0.08)				5
Liaoning	Shenyang (40)	2013-2014	23 279	2	1/11 640			0: 2	2: 0	8
Hubei	Shiyan (41)	2016-2017	70 937	3	1/23 646	308(0.43)	299(97.08)			6
Shaanxi	Baoji (42)	2011-2015	192 469	5	1/38 494					9
Fujian	Fuzhou (43)	2013	15 136	1	1/15 136	76(0.50)	70(92.11)			5
Yunan	Kunming (44)	-2007	11 791	2	1/5 896					6

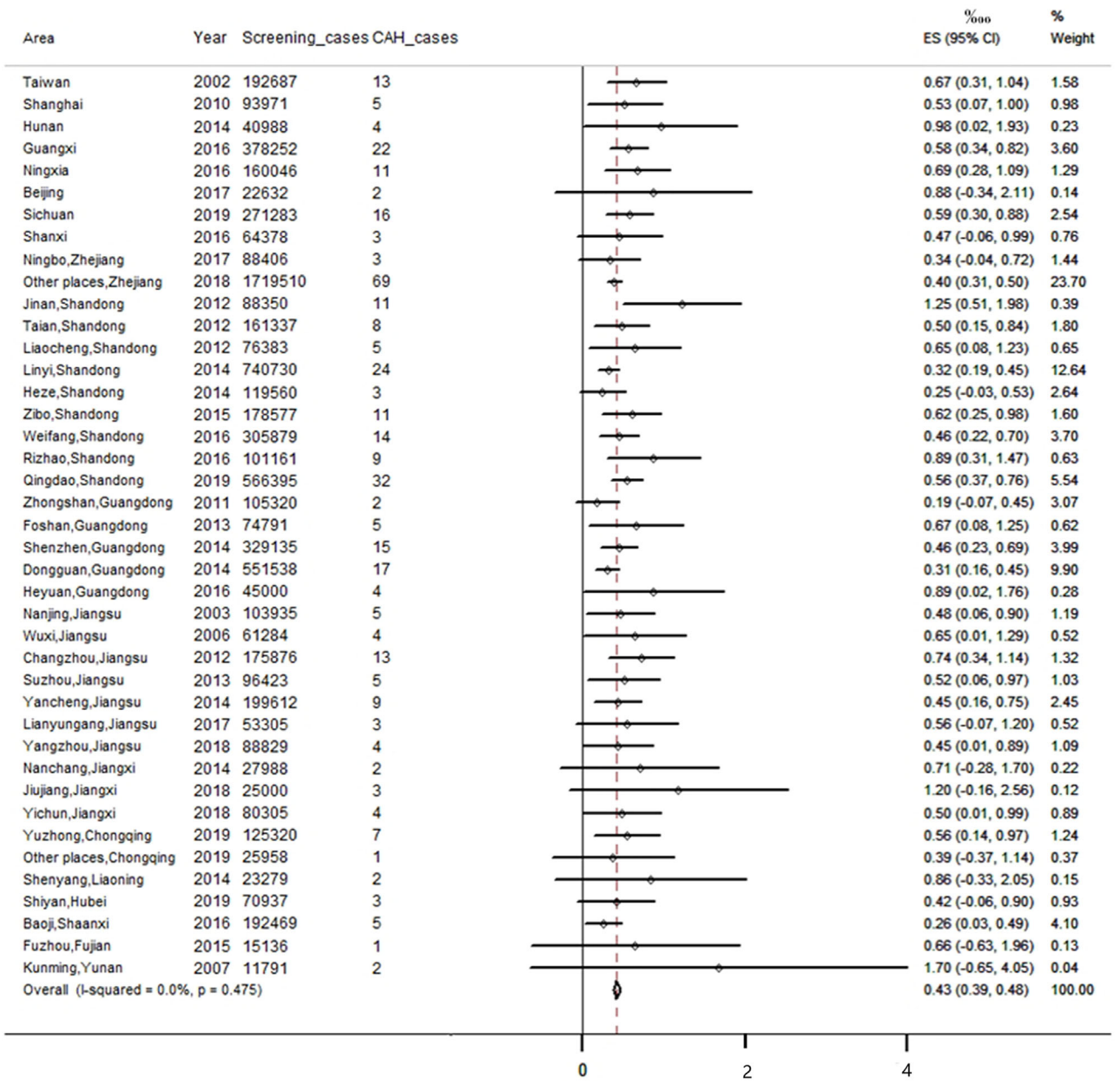

FIGURE 2 | Meta-analysis of CAH incidence in different regions of China.

CAH (see **Table 1** and **Figure 3**). Of the screened newborns, 95% (except part of Ningxia and Sichuan province) were located to the east of the Heihe-Tengchong line (an imaginary line that divides the area of China into two roughly equal parts with contrasting population densities; west of the line: 57% of the area, but only 6% of the population; east of the line: 43% of the area, but 94% of the population). Among them, the sex ratio of the screened newborns described in our studies was 1.10:1 (1 678 399: 1 527 300). We found that the ratio of males to females with CAH described in some studies was 1.92:1 (119:62), while the ratio of SW to SV type was 3.25:1 (104:32). The average level of 17-OHP (n=74) for patients diagnosed with CAH was 393.40 ± 291.85 nmol/L (Range 33-1 300 nmol/L), there was no significant difference between patients of different genders [male(n=36): 437.17 ± 297.27 nmol/L (Range 33-1 300 nmol/L) v.s. female (n=22): 322.25 ± 293.04 nmol/L (Range 33.2-1 040 nmol/L), P=0.16], but a statistical difference was found between SW and SV type [SW(n=25): 483.29 ± 330.07 nmol/L(Range 48-1 300 nmol/L) v.s. SV(n=3): 73.80 ± 7.83 nmol/L (Range 65-80 nmol/L), P=0.04].

Results of Meta-Analysis

Incidence of CAH

In the included studies, 41 studies reported the incidence of CAH. Since there was no evidence of significant heterogeneity among the studies (I^2 = 0%, P<0.05), a fixed-effect model was used for analysis. The result of meta-analysis showed that the incidence of CAH was 0.43‰ [95%CI, (0.39‰, 0.48‰)], or 1/23 024 [95%CI, (1/25 757,1/20 815)]. We also performed a subgroup analysis of regional incidences, among them, the incidence in Zhejiang, Guangdong, Hubei and Shaanxi province was lower than the national incidence; but in other regions, it was higher than the national incidence (see **Figures 2** and **3**).

Screening Positive Rate

In the included studies, 27 studies reported the positive rate of CAH screening. We found that 3 985 456 newborns were screened in these studies and 23 925 cases were considered as suspected positive cases. As I^2> 50%, we used a random effect model for analysis. The result of the meta-analysis showed that the positive rate of CAH screening in China was 0.66% [95%CI, (0.54%, 0.78%)] (see **Figure 4**).

Recall Rate of Positive Cases

In the included studies, 17 studies reported the recall rate of suspected positive cases. We found that 20 158 suspected positive cases were considered in our studies and 17 861 cases were successfully recalled, among which 135 cases were diagnosed with CAH (positive predictive value: 0.76%). As I^2> 50%, we used a random effect model for analysis. The result of the meta-analysis showed that the recall rate of positive cases in China was 86.17% [95%CI, (82.70%, 89.64%)] (see **Figure 5**).

Publication Bias Across Studies

Publication bias was shown by a funnel plot and evaluated by the Begg's test using Stata 12.0 software. As for the main indicator (the incidence of CAH), the funnel plot showed that all the included studies were symmetrically distributed in the triangle area (see **Figure 6**), which meant that they were less affected by publication bias. Begg's test showed P=0.204 for the incidence of CAH, as for the other indicators, no publication bias was found between them (P value of the positive rate and the recall rate were 0.868 and 0.902, respectively).

DISCUSSION

Our meta-analysis included 41 studies on CAH screening of newborns in China, including approximately 7.85 million newborns, which is the most comprehensive and systematic

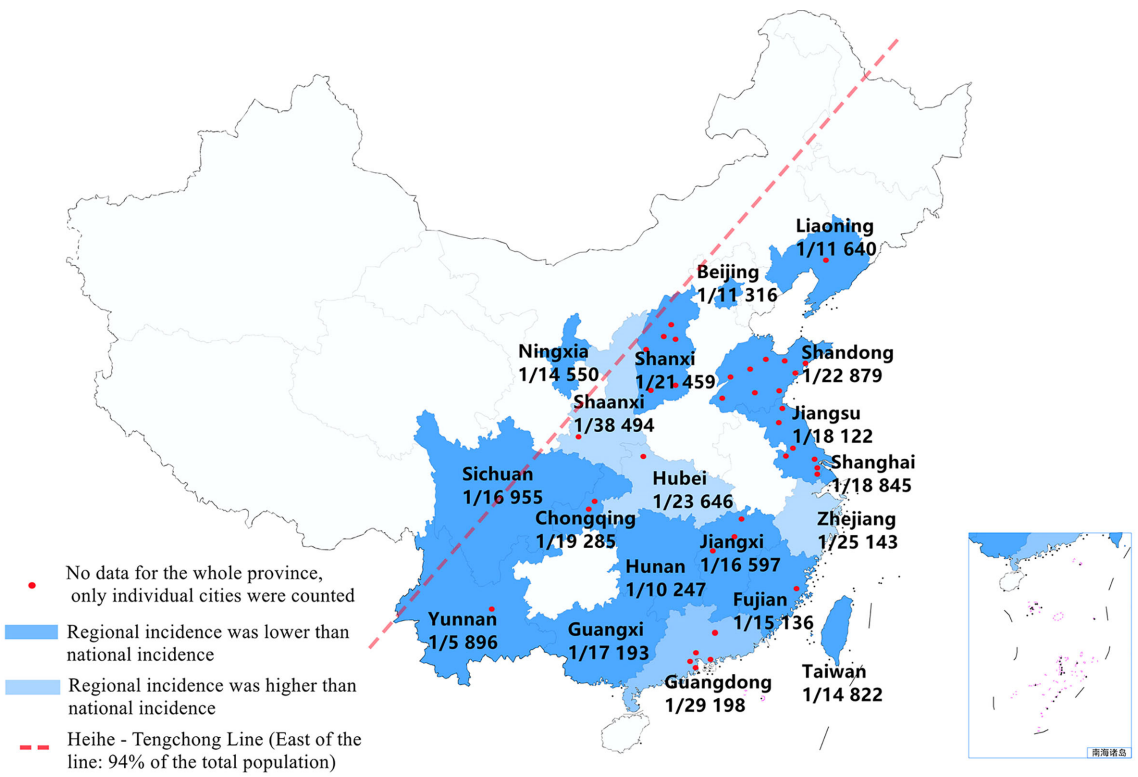

FIGURE 3 | The schematic diagram shows the incidence of CAH in different provinces of China (provincial data obtained by subgroup analysis). Note: Of the screened newborns, 95% cases (except part of Ningxia and Sichuan province) were located to the east of the Heihe-Tengchong line (an imaginary line that divides the area of China into two roughly equal parts with contrasting population densities; west of the line: 57% of the area, but only 6% of the population; east of the line: 43% of the area, but 94% of the population).

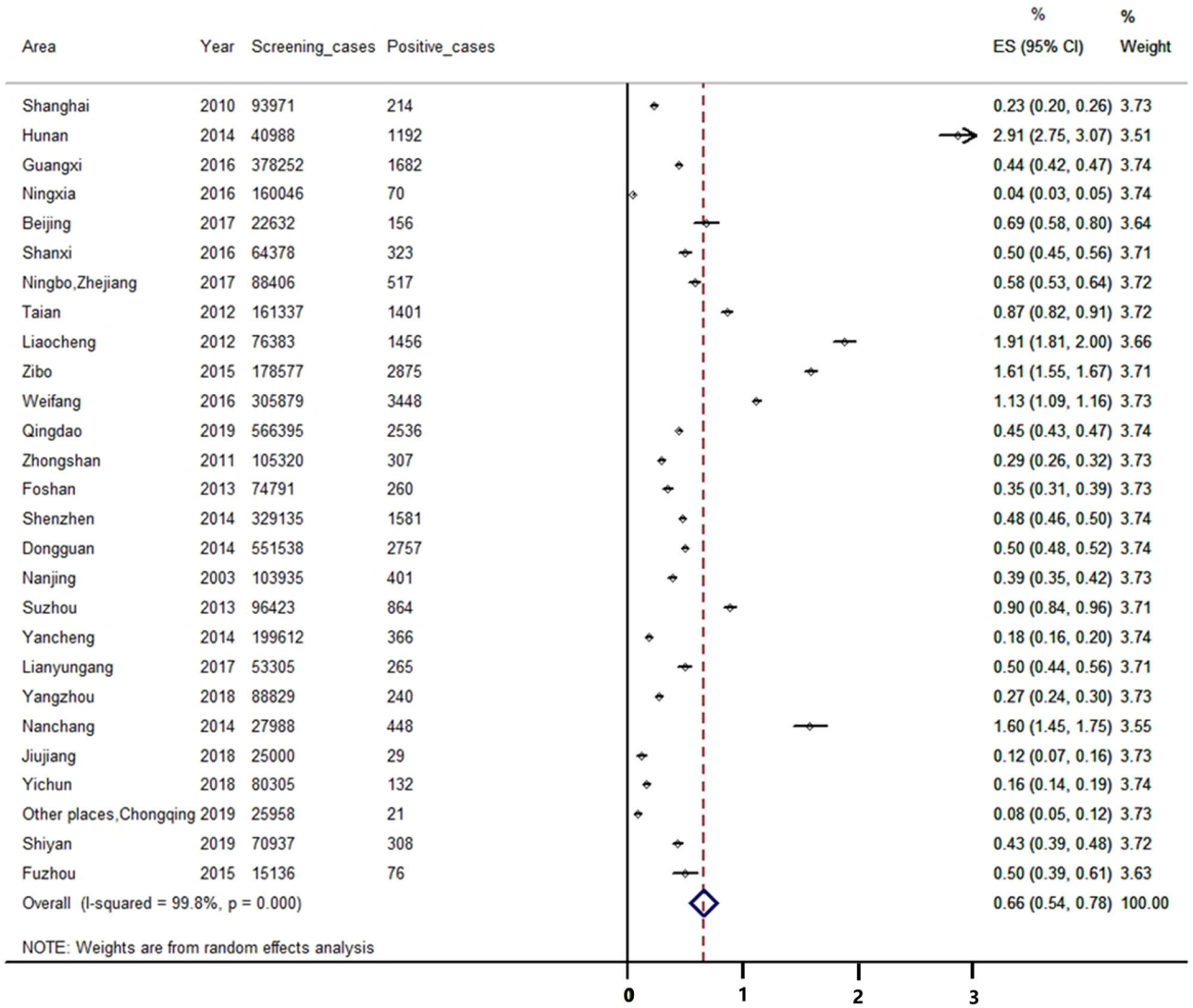

FIGURE 4 | Meta-analysis of the positive rate of CAH screening.

analysis of CAH screening in the world. Due to the large sample size, representative population distribution, and no publication bias in the literature, the results of our study are objective and reliable.

According to the degree of enzyme deficiency, classical CAH represented by 21-OHD can be divided into two types: SW (about 75%) and SV (about 25%) type. In our study, we found that the ratio of SW to SV type is 3.25:1, which is consistent with previous literature reports. Due to the almost complete lack of enzyme activity, SW type will present with more typical clinical symptoms, just as in our study, 17-OHP levels of SW type were significantly higher than that of SV type. Theoretically, the incidence of autosomal recessive genetic disease such as CAH is the same for males and females. However, it's interesting that the CAH screening results in our study showed that the incidence of CAH in males was much higher than that in females (1.92:1). Such a difference has not been reported elsewhere, so we need to be cautious about this result. Possible explanations based on China's national conditions need to be considered: Firstly, there was a serious gender imbalance in China, and the screening data also showed the proportion of males was much higher than that of females (1.10:1); and Chinese parents will pay more attention to boys, such that the recall rate of positive boys may be higher than that of girls. Another explanation might be that some females were clinically diagnosed due to ambiguous genitalia after birth or even during pregnancy, making sample screening unnecessary (72 hours after birth). Also, since male patients with CAH tend to have higher levels of 17-OHP than the female, they may be more sensitive to CAH screening. A study of 220 000 newborns in the United States (45) showed that the sensitivity of newborn screening for male infants is 80%, while the female is only

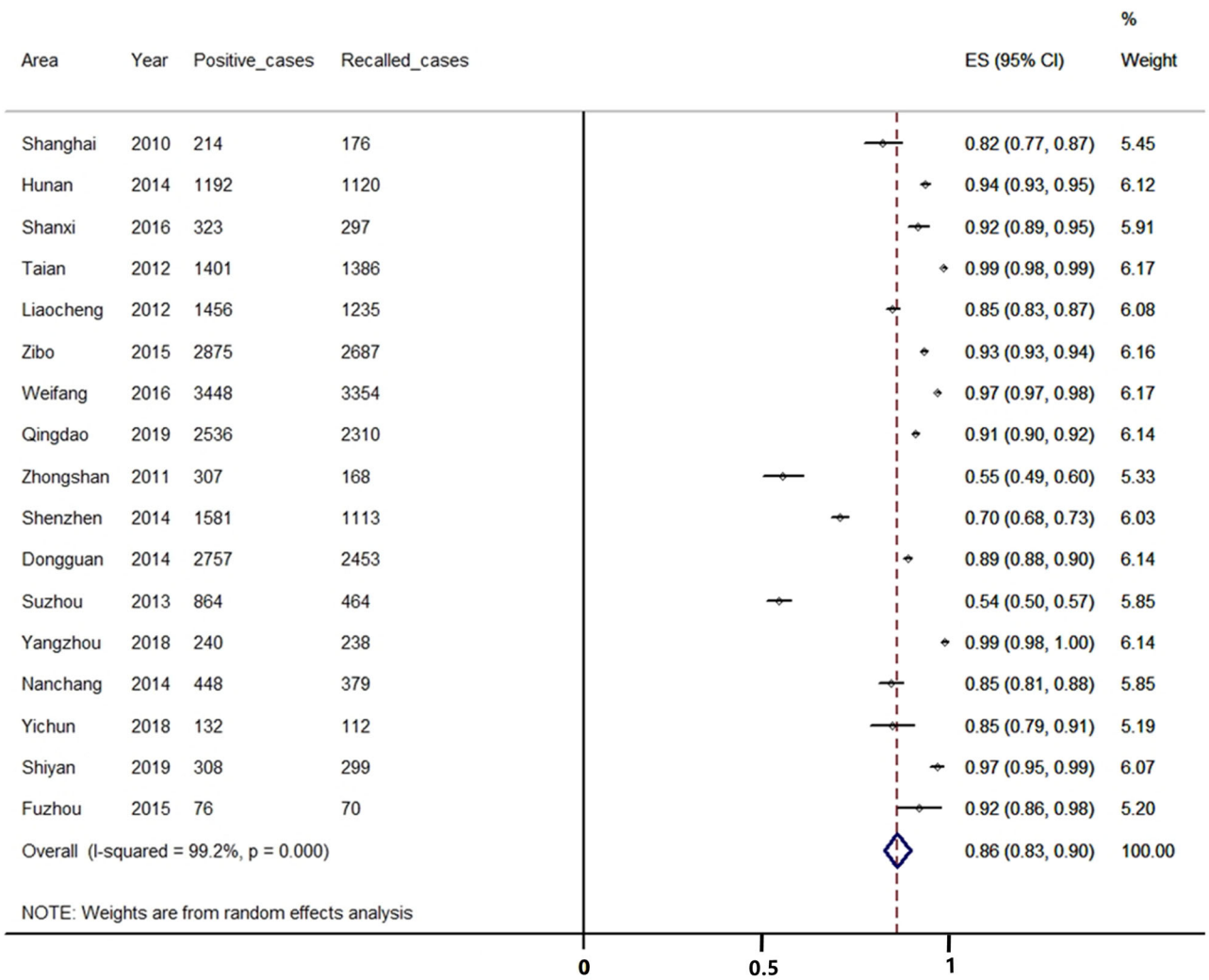

FIGURE 5 | Meta-analysis of the recalled rate of positive cases during CAH screening.

60%. These reasons may account for the differences in our study results compared with previously reported studies, but we have to interpret these results objectively. Large-scale and prospective research will help verify our analysis.

According to relevant screening statistics, there are obvious racial and regional differences in the incidence of CAH. The global incidence of CAH is about 1/14 000-1/18 000 (2), among which, Japan is 1/19 859 (46), New Zealand is 1/26 727 (47), France is 1/15 699 (48), and Sweden is 1/14 260 (49). Our study has shown that the incidence of CAH in China is 0.43‰ (0.39‰, 0.48‰), that is 1/23 024 (1/25 757, 1/20 815), which is lower than most countries in Europe and America, and close to Asia-Pacific countries such as Japan and New Zealand. In China, the incidence in Zhejiang, Guangdong, Hubei and Shaanxi province is lower than the national incidence; but in other regions, it is higher than the national incidence. However, because of the inequality in medical provision, particularly the under-developed health-care in western China, missed diagnosis and misdiagnosis may occur, which may render the incidence of CAH screening lower than the actual incidence.

At present, the DELFIA or ELISA method is extensively used in China to detect the 17-OHP concentration in dried blood spots for CAH screening. These methods have strong specificity and high sensitivity, and provide a good technical accuracy for the screening work. In our study, 27 studies included 3 985 456 newborns reported the positive rate of CAH screening, among which 23 925 cases were considered as presumptive positive cases. Our study found that the positive rate (0.66%) of primary screening for CAH was much higher than the incidence rate (0.43‰), meaning that the current screening method may have a high false positive rate and a low positive predictive value. 17-OHP is a sensitive indicator for screening for CAH, but the setting of an appropriate cut-off value is difficult especially in premature and low birth weight infants which may give controversial screening results. Secondary screening such as liquid chromatography-tandem mass

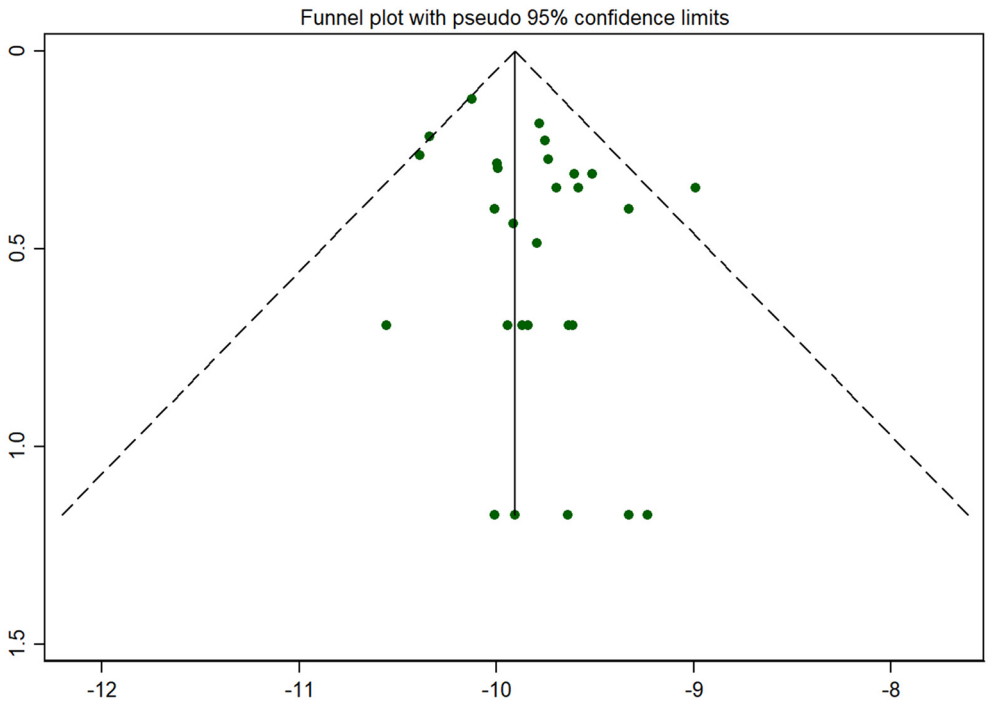

FIGURE 6 | Funnel plot for publication bias. Note: As for the main indicator (the incidence of CAH), the funnel plot showed that all the included studies were symmetrically distributed in the triangle area, which meant that they were less affected by publication bias.

spectrometry (LC-MS/MS) can greatly improve the sensitivity and specificity of CAH screening. For example, within 3 years of using LC-MS/MS as secondary screening, the positive predictive value of the CAH screening in Minnesota, the United States, increased from 0.64% to 7.3%. However, LC-MS/MS can be used only as a supplement to primary screening and cannot completely replace the current methods.

In addition, compared with screening for congenital hypothyroidism and phenylketonuria, the screening coverage and recall rate of CAH are still very low. Our study included 17 studies, 20 158 suspected positive cases were considered, but only 17 861 cases were successfully recalled. Meta-analysis showed that the recall rate was only 86.17% (82.70%, 89.64%). This suggests that about 14% of newborns with positive results failed to be recalled, and there was a risk of delayed diagnosis or even missed diagnosis. The Southeast region accounts for the vast majority of China's population, but the recall of newborns may be hampered by the complex population structure in southeast China, which has a large number of migrants and high mobility. We believe that because of the low awareness of some screening institutions and insufficient diagnostic level of some underdeveloped areas, CAH screening and diagnosis may be limited. Therefore, we should endeavor to raise public awareness of CAH to improve cooperation with the CAH screening program.

CONCLUSION

Through the systematic analysis of the results of CAH screening for newborns in China, we have obtained a relatively accurate incidence of CAH in China (1/25 757, 1/20 815). In addition, we have established some interesting clinical characteristics of CAH, such as the ratio of different types and gender of CAH as well as their 17-OHP levels, which will provide valuable data for the screening and diagnosis of CAH in the future. However, we also realize that there are still some problems with CAH screening at present, such as the insufficient screening coverage in China, the difficulty of recalling positive cases, the imperfect setting of the 17-OHP cut-off value and the low positive predictive value, which will guide our future work in CAH neonatal screening. In summary, our study involving the largest number of babies on the incidence and regional characteristics of CAH provides data which suggest that improving laboratory testing capacity and equity of the CAH screening service throughout China should improve survival and quality of life for all.

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

YL conceptualized and designed the study. ZL supervised the data collection, reviewed the analyses and wrote all

versions of the manuscript. LH, CD, CZ, MZ and XL coordinated and supervised data collection, critically reviewed the manuscript and approved the final manuscript as submitted. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2021.624507/full#supplementary-material

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frontiers in Pediatrics

MINI REVIEW
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Hyperglycaemia in the Newborn Infant. Physiology Verses Pathology

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Hyperglycemia is common in newborns requiring intensive care, particularly in preterm infants, in sepsis and following perinatal hypoxia. The clinical significance, and optimal intervention strategy varies with context, but hyperglycaemia is associated with increased mortality and morbidity. The limited evidence for optimal clinical targets mean controversy remains regarding thresholds for intervention, and management strategies. The first consideration in the management of hyperglycaemia must be to ascertain potentially treatable causes. Calculation of the glucose infusion rate (GIR) to insure this is not excessive, is critical but the use of insulin is often helpful in the extremely preterm infant, but is associated with an increased risk of hypoglycaemia. The use of continuous glucose monitoring (CGM) has recently been demonstrated to be helpful in targeting glucose control, and reducing the risk from hypoglycaemia in the preterm infant. Its use in other at risk infants remains to be explored, and further studies are needed to provide a better understanding of the optimal glucose targets for different clinical conditions. In the future the combination of CGM and advances in computer algorithms, to provide intelligent closed loop systems, could allow a safer and more personalized approached to management.

Keywords: hypoxic ischaemia, hyperglycaemia, preterm, glucose, hypoglycaemia, monitoring

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INTRODUCTION

Although *in utero* glucose levels are normally maintained between 4 and 6 mmol/l hyperglycaemia is common in newborns requiring intensive care, particularly in preterm infants, in sepsis and following perinatal hypoxia (1, 2). Transient hyperglycaemia may be a physiological response to stress but when prolonged is associated with significant morbidity and mortality. Hyperglycaemia has variably been defined based on absolute thresholds, as well as length of exposure and association with glycosuria. Threshold definitions range from >7 to >13.3 mmol/l (>126–239 mg/dl) (3–6). The most common definition is blood glucose (BG) >10 mmol/l (180 mg/dl) (3). However, the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recommends avoiding glucose levels >8 mmol/l (145 mg/dl), because they are associated with increasing morbidity and mortality (7). Hyperglycaemia is most commonly seen in the extremely preterm infant in the first week of life, reports varying between 20 and 86% (1, 8–17). However, glycaemic instability and hyperglycaemia remain in these infants even at the time of discharge (1, 8–16). Hyperglycaemia is also prevalent in infants following hypoxic ischaemic (HI) insults (18), associated with sepsis and in neonatal diabetes which has recently been reviewed (19). The use of systemic steroids, inotropes, and caffeine (20, 21), as well as stress associated with intubations can also increase glucose levels (1).

The limited evidence for optimal clinical targets mean controversy remains regarding thresholds for intervention and management strategies. The first consideration in the management of hyperglycaemia must be to ascertain potentially treatable causes. Hyperglycaemia in the newborn may be initially considered an acute catabolic response to stress, but prolonged hyperglycaemia is associated with a poor prognosis. There are numerous studies reporting its association with increased morbidity and mortality, and there are biologically plausible causal pathways. These include the direct effects of hyperglycaemia *per se*, as well as the effect of relative insulin deficiency. As hyperglycaemia is an easily modifiable risk factor for poor outcomes it is important to understand potential mechanisms of harm, and intervention strategies that could improve outcomes.

HYPERGLYCAEMIA IN THE PRETERM INFANT

The prevalence of hyperglycaemia is inversely related to gestational age with extremely preterm infants at most risk. Many preterm morbidities as well as mortality are associated with increased hyperglycaemic exposure (22). These include retinopathy of prematurity (23), chronic lung disease (24), necrotizing enterocolitis (NEC) (25), hypernatraemia, and reduced white matter in the brain at term (26). Associations are often reduced or lost after adjusting for gestational age and birth weight, as there is a close relationship between hyperglycaemia and immaturity. It is similarly difficult to separate how much hyperglycaemia is a marker of the metabolic disturbance, that is the primary etiology for poor outcome, as opposed to contributing itself to that causal pathway. However, even after adjustment for gestational age, early hyperglycaemia has been shown to be associated with an increased risk of death or sepsis, OR 5.07 (95% CI 1.06–24.3) (27). Furthermore, hyperglycaemia has been associated with poor growth up to 2 years of age (28, 29). The implications of hyperglycaemia and prolonged catabolism on longer term metabolic and neurocognitive outcomes for preterm infants remains to be determined.

Pathogenesis of Hyperglycaemia in the Preterm Infant

In preterm infants hyperglycaemia can be considered to result from a combination of excess glucose delivery, counter regulatory response to stress and infection, and the impact of prematurity and growth restriction on insulin secretion and sensitivity (12, 30, 31).

Central and Peripheral Glucose Insensitivity and Insulin Resistance

In the healthy adult glucose infusions (6 mg/kg/min) completely suppress endogenous glucose production. However, in the preterm neonate glucose production is not suppressed in the same way by glucose infusions. Studies in the newborn have shown glucose levels can reach >13.9 mmol/l (250 mg/dl), or glucose infusion rates (GIRs) >16 mg/kg/min before glucose production is suppressed (32–34). Similarly large reductions in the GIRs may not alter glucose production rates, when one might anticipate it would lead to an increase in gluconeogenesis (35). Persistent endogenous glucose production, in spite of glucose infusion, has been shown in preterm infants even at the age 2–5 weeks (36). This may in part be due to immature expression of glucose transporters (GLUT), particularly glucose transporter 2 (GLUT2) and glucose transporter 4 (GLUT4). Low GLUT 2 levels in the liver may lead to lack of glucose sensitivity, and continued hepatic glucose production (37). The less abundant insulin sensitive tissues (adipose and skeletal muscle), and low GLUT4 expression in muscle, may also result in reduced insulin mediated glucose uptake in preterm infants (2, 38). Increased levels of pro-inflammatory cytokines (tumor necrosis factor-α, interleukin-1, interleukin-6), secondary to chorioamnionitis, sepsis or NEC, may also lead to insulin resistance, and altered insulin receptor signaling. Intensive care interventions, such as the use of inotropes and corticosteroids, also increase insulin resistance and suppress insulin secretion.

Relative Insulin Deficiency

In utero studies suggest that insulin levels increase toward term, and immaturity of the β-cells may result in insufficient insulin secretion (6). GLUT 2 transporters are involved in glucose stimulated insulin secretion from the pancreas, but fetal pancreatic β cells do not express GLUT 2 until 7 months (39, 40), impacting on the β cell's response to hyperglycaemia (32). In the preterm infant the insulin secretory response to glucose is reduced compared with the term infant, but increases postnatally over a number of weeks (41). Preterm infants are often also growth restricted, and this is associated with reduced β cell mass (42, 43). However, these changes are dependent on the model and timing of growth restriction (44, 45). The levels of proinsulin (a less active precursor to insulin), are high in preterm neonates, suggesting that the processing of proinsulin in β-cells is partially defective. This relative insulin deficiency may contribute to reduced insulin like growth factor 1 (IGF-I) generation, with further impacts on metabolism and growth.

Feeding and Incretins

Incretins play an important role in augmenting insulin secretion in adults (46, 47), and the delay in enteral feeding of preterm infants means the normal stimulation of incretins does not occur (48). In the preterm infant glucose control often improves once enteral feeds have been established, but even when enteral feeds are given, preterm infants do not demonstrate an equivalent incretin response compared to that seen in term infants (49).

Clinical Consequences of Hyperglycaemia

An initial counter regulatory response and transient hyperglycaemia may be beneficial in acute stress, and may be considered physiological. However, prolonged hyperglycaemia in critical illness has been associated with poor prognosis. Furthermore, for the preterm infant, the period from birth to term is a critical period of development, and even shorter periods of hyperglycaemia may be harmful. It remains unclear as to whether harmful effects of dysglycaemia are mediated by the

primary effects of hyperglycaemia per se, or the effects of relative insulin deficiency, with both potentially having short and long term clinical consequences.

Primary Role of Hyperglycaemia

Hyperglycaemia is harmful to cells and can lead to an over expression of insulin independent glucose transporters (GLUT-1, GLUT-2, and GLUT 3), which leads to an increase in glucose uptake by endothelial, hepatic, immune, and nerve cells (50). Glucose overload can cause an increased generation of oxygen free radicals, which can cause mitochondrial dysfunction and increased apoptosis. Hyperglycaemia also impairs leukocyte phagocytic function, decreases complement function, increases pro-inflammatory cytokines, and impairs neutrophil chemotaxis, all leading to an increased susceptibility to infections (51–54).

Independent manipulation of BG and insulin levels in an animal model of hyperglycaemia (burn injured parenterally fed rabbit), demonstrated survival to be better in the normoglycaemic groups (89% verses those with hyperglycaemia 53–64%) (55). Recent data have also shown a causal pathway linking hyperglycaemia to an increased risk of microbial gut translocation in both animal models and adult studies (56). Persistent hyperglycaemia has been associated with NEC, OR 9.49 (95% CI 1.52–59.3) and infection, OR 3.79 (1.40–10.20) (1, 25, 27) which are two major causes of mortality and morbidity for preterm infants (57, 58).

Hyperglycaemia can lead to an osmotic diuresis, hypernatraemia, and electrolyte imbalance and has been associated with intraventricular hemorrhage (IVH) (25, 59, 60). More significant may be the impact of hyperglycaemic on nervous system development and injury in animal models (61). Hyperglycaemia increases central nervous system permeability, oxidative stress, and leads to microglia activation and astrocytosis, as well as regulation of DNA repair mechanisms, compromising neuronal and glial cell integrity (62). This can lead to long-term changes in synaptogenesis and behavior (63). Clinical correlates include the finding in a cohort of extremely preterm infants that hyperglycaemia >8.3 mmol/L(150 mg/dl) on the first day of life was an independent risk factor for white matter reduction on term MRI (26). Increased BG concentrations have also been associated with decreased total absolute band power on EEG, a measure expressing background brain activity, and associated with long term outcomes (64). A large retrospective study, (including 443 preterm infants) showed hyperglycaemia to be associated with lower survival without neurodevelopmental disability at 2 years of age, but this did not remain significant after adjusting for gestational age, birth weight z-score, and socioeconomic status (65). However, the close relationship between hyperglycaemia and gestational age make separation of the causal effect of hyperglycaemia from that of immaturity challenging. Data in press from the Swedish EXPRESS cohort shows that hyperglycaemia is associated with worse motor outcomes in early childhood (after multivariate adjustment). Further long term follow up studies are required to explore the long term impact of hyperglycemia per se, and different management strategies.

Impact of Relative Insulin Deficiency

Hyperglycaemia may also be considered a marker of relative insulin deficiency, and this may have independent effects to those of hyperglycaemia. In both animal and human models insulin has been shown to improve innate immunity, and to suppress pro-inflammatory products, whilst increasing anti-inflammatory cytokines (66–70). Insulin deficiency may be associated with reduced expression of nitric oxide synthase (iNOS), and insulin may be protective by prevention of excess nitric oxide release. Insulin can also improve cardiac function (55), and in patients post myocardial infarction and in sepsis, the combination of glucose and insulin infusion improves cardiac function (71). Insulin infusions can reduce proteolysis, and in burns have a positive impact on protein synthesis and wound healing (72–74). Relative insulin deficiency can also lead to low IGF-I levels, which can be detrimental, as IGF-I is an important mediator of growth in the neonatal period. Starvation and critical illness lead to suppression of IGF-I levels, and IGF-I administration has been shown to increase nitrogen balance in catabolic states (75, 76). IGF-I is also an important growth factor influencing perinatal pancreatic development, with low levels leading to increased apoptosis, and potentially resulting in reduced β-cell mass. Therefore, insulin deficiency has implications in the preterm infants for growth, as well as longer term metabolic health.

Clinical Interventions for the Management of Hyperglycaemia

Thresholds for intervention remain controversial, but the recent ESPGHAN and ASPEN guidelines clearly advise avoiding glucose levels >8 mmol/l (145 mg/dl) (7), or >8.3 mmol/l (150 mg/dl) (77, 77). Approaches to management should always involve reviewing the context of hyperglycaemia, with particular consideration as to whether there is evidence for acute illness, such as infection which requires treatment. Limitation of excess glucose intake should then be considered, and insulin used in the context of wishing to maintain postnatal growth when appropriate (4). Simply increasing calorie intake may be detrimental (14), but optimizing amino acid intakes and the use of insulin has the theoretical potential to improve lean body mass, and pancreatic function (78). The potential benefits of insulin however need to be balanced with the risks of hypoglycaemia, and therefore careful monitoring of glucose levels should be undertaken on any infant on insulin.

The Importance of Parenteral Nutrition

The relationship between glucose infusions and hyperglycaemia is not consistent. Some studies have shown a direct positive relationship between GIRs and risk of hyperglycamia, other studies have not found such a clear relationship (1, 20, 60, 79, 80). Differences may relate to the rates of glucose being infused, with excess rates clearly associated with hyperglycaemia, but the impact of lower rates of glucose infusion more nuanced. At lower GIRs the influence of differences in other components of parenteral nutrition (PN) may play an important role. Amino acids stimulate insulin secretion, and low plasma arginine levels have been associated with hyperglycaemia (81). Neonates receiving amino acid infusions in addition to glucose have

higher insulin levels (82). A Cochrane meta-analysis concluded that higher amino acid intake in PN was associated with a reduction in hyperglycaemia. Therefore, reducing PN al intake may be counterproductive, if it reduces amino acid intake along with glucose load. One study that reported on the impact of a change in clinical practice, aimed at limiting dextrose intake (to minimum of 4 mg/kg/min), demonstrated a reduction in the prevalence of hyperglycaemia, use of insulin and mortality. However, total protein and energy intakes were also higher after the intervention, which cannot therefore be viewed as a simple intervention on dextrose intake (83). Lipid infusions may have a beneficial effect, by reducing the glucose load whilst maintaining energy intakes, they can reduce the prevalence of hyperglycaemia (84). However, in excess or in acute illness, lipids have been reported to contribute to hyperglycaemia (85).

A single center study in Norway showed implementation of an enhanced PN protocol was associated with an increased prevalence of severe hyperglycaemia, and higher mortality (14). However, in the multivariate analysis, the enhanced PN regimen per se was not predictive of mortality, it was the early severe hyperglycaemia that was the strongest risk factor for death. After adjusting for potential confounding variables, early severe hyperglycaemia was an independent risk factor for death (OR, 4.68; 95% CI, 1.82–12.03), greater than that of gestational age (odds ratio, 0.62; 95% CI, 0.49–0.79). Attempts to reduce the prevalence of hyperglycaemia, by controlling glucose intake, have included the use of continuous glucose monitoring (CGM) (86). In this study, glucose delivery was determined by a computer guided GIR that was supported by either real time CGM (intervention), or intermittent BG levels (control). Those in the intervention arm (using CGM), showed an increased median time in target (72–144 mg/dL, 4–8 mmol/l), of 84% compared to 68% in controls.

There are good reasons to ensure that excess glucose delivery is avoided, as exceeding maximum glucose oxidation rates can cause increased carbon dioxide production, lipogenesis and fat deposition including liver steatosis (87). High rates of glucose infusion and hyperglycaemia can themselves lead to increased insulin resistance and endogenous glucose production (88). In appropriately grown preterm newborns the maximum rate of glucose oxidation has been estimated to be 6–8 mg/kg/min, compared to term infants, and infants on long term PN where maximum glucose oxidation rates are 12 mg/kg/min (89). When determining glucose requirements, and optimal management for hyperglycaemia it is important to consider the metabolic phase of illness. During the acute phase of critical illness, such as sepsis, increasing glucose and nutritional intake will not promote anabolism and may be detrimental (90). In contrast, in a more stable preterm infant, where growth and anabolism are the priority, the approach to hyperglycaemia would normally be to favor optimizing nutritional delivery. ESPGHAN recommend parenteral glucose intake of 4–8 mg/kg/min on day 1 (and during any subsequent acute illness such as infection), rising to 8–10 mg/kg/min over the subsequent 2–3 days to allow for growth (7). Both ESPGHAN and the American Society for Parenteral and Enteral Nutrition recommends maintaining GIRs (<12 mg/kg/min), but not reducing to <4 mg/kg/min (77). If hyperglycaemia persists (>10 mmol/l, 180 mg/dL), it is then recommended that insulin treatment should be started (7).

The Role of Insulin

A number of small single center studies suggest that the use of insulin can help to maintain nutritional intake. These studies showed that infants who were hyperglycaemic, and randomized to treatment with insulin, tolerated higher GIRs, and had greater weight gain, in comparison to those treated with reduced glucose intake, who remained catabolic for longer (91–97). These findings may be related to a decrease in proteolysis, but also protein synthesis. One small study raised concern that insulin infusions significantly increased lactic acidosis, and did not impact on protein synthesis. However, this study infused high rates of glucose (14–17 mg/kg/min) without the infusion of any amino acids (98).

There are limited data from larger interventional studies in the preterm newborn. The NIRTURE Trial, a large multicentre randomized controlled trial used early insulin treatment prior to the onset of hyperglycaemia, with the aim of promoting anabolism. The trial did not demonstrate benefits and was stopped early on the grounds of futility. The study was important though in highlighting the high prevalence of clinically "silent" hypoglycaemia in both study arms. These data were achieved by uniquely collecting data on glycaemic exposure using CGM (blinded to the clinical team) and raised concerns about the challenges of insulin treatment (13).

The use of insulin to achieve "tight" glucose control has been widely debated since the landmark paper of van den Berghe which showed dramatic improvements in adult intensive care outcomes in patients randomized to tight glucose control (99). Many studies trying to replicate the positive findings of this study have raised concerns about, or been stopped early, due to the risk of severe hypoglycamiaemia (100, 101). The largest adult study showed increased risk of death in the intensive study arm (OR 1.14; 95% CI 1.02–1.28; $P = 0.02$), but highlighted the association of hypoglycaemia with mortality (102). In this context tight glucose control refers to glucose levels being maintained within a much narrower "normoglycaemic" range (typically 4–6 mmol/l), than standard care which aims to prevent hyperglycaemia (typically >8–10 mmol/l). Further differences between studies have been highlighted including: underlying reason for patients requiring intensive care, ability to achieve target levels of control and the early use of PN (103, 104). The use of PN having been shown, more recently, to be harmful, in adult and PICU. Preplanned analyses in these studies showing harmful effects being related to aminoacids, but not glucose or lipids (105). There are important differences in the preterm infant in NICU, compared with the adult in ITU, in relation to the importance of growth on survival, and differences in prevention of hyperglycaemia compared to tight glucose control.

The HINT trial is the only trial to explore tight glycaemic control in preterm infants. In this study the intervention arm "tight control" targeted glucose levels of 4–6 mmol/L (72–108 mg/dL), compared to the unit standard of care which was 8–10 mmol/L (144–180 mg/dL). The study showed high rates of hypoglycaemia in both study arms and variable effects on growth

parameters (106). The study reported no overall effect on survival without neurodevelopmental delay, intelligence scores or motor skills at seven years of age, although there was beneficial effect in those who actually reached the target of 4–6 mmol/L (72–108 g/dL), but power was limited for assessing such outcomes. The effects of hypoglycaemia also had the potential for masking any effects of prevention of hyperglycaemia. More recently a large study from the National Swedish EXPRESS Cohort demonstrated, insulin treatment of hyperglycaemia in the first 28 days of life, was associated with lower 28- and 70-day mortality (17). However, in this retrospective study there were no clear criteria either for starting or modifying insulin therapy, or fixed glucose target within the different study sites.

Challenges in insulin treatment in preterm babies relate to the combination of rapidly changing insulin sensitivity, the difficulty of consistent insulin delivery, and the low frequency of glucose monitoring. Hyperglycaemia itself causes insulin resistance and following increasing insulin to regain normoglycaemia, insulin requirements often fall, and this increases the risk of hypoglycaemia (13). Insulin is easily adsorbed onto intravenous lines, and the use of large volume syringes for delivery at small infusion rates makes insulin delivery unpredictable (107, 108). Monitoring of glucose levels in preterm infants is often infrequent, and studies using CGM have shown that real time CGM alone, or in combination with computer algorithms, has the potential to reduce the prevalence of hyperglycaemia without increasing the risk of hypoglycaemia (91, 109). Furthermore, a recent international multicentre trial has demonstrated that the use of CGM in preterm infants can safely support the targeting of glucose control without causing hypoglycaemia, and is cost effective (110, 111). However, optimal target glucose levels remain to be determined.

Hypoxic Ischaemic Encephalopathy

Both hyperglycaemia and hypoglycaemia are common in babies following perinatal HI insult. The etiology of hyperglycaemia following HI, in comparison with that of the preterm infant, is predominantly driven by the effects of stress hormones and tissue damage from hypoxia. Although hypoglycaemia has traditionally been considered a more significant risk, there is increasing evidence that hyperglycaemia is a modifiable mediator of long-term morbidity (18). Hyperglycaemia is reported in 50% of babies using intermittent BG testing, and CGM has revealed that exposure to hyperglycaemia is often more frequent and prolonged (112, 113). Pediatric intensive care studies have also shown longer duration, higher peak glucose levels, and increased glucose variability are all associated with mortality and morbidity (114).

In the analyses of the cool cap study, a multicenter trial of cooling for HIE, hyperglycaemia was confirmed as an independent risk factor for poor outcomes at 18 months (18). Further *post-hoc* analyses, after adjusting for Sarnat stage and 5 min Apgar score, only hyperglycaemic infants randomized to hypothermia had reduced risk of death and/or severe neurodevelopmental disability at 18 months (adjusted risk ratio: 0.80, 95% CI 0.66–0.99). This suggests that early glycaemic profile in infants with moderate-to-severe HIE identifies those at most risk of multi-organ dysfunction and most likely to benefit from therapeutic hypothermia (115). In neonates with encephalopathy, even after adjusting for hypoxia-ischemia severity, epochs of hyperglycaemia were associated with worse neural injury, as well as global brain function and seizures (116, 117). Whether hyperglycaemia causes neuronal injury or is simply a marker of severe brain injury is yet to be determined (116, 117).

Many potential causal mechanisms have been implicated in infants with HIE: dyslipidemia, inflammatory cytokine production, endothelial dysfunction, hypercoagulation, glucose toxicity, increased cellular apoptosis, and over-production of superoxide. However, there are potential differences in impact related to maturity of the newborn nervous system compared to similar ischaemic injuries in adults (118). Deleterious effects on the nervous system may be related to increased hyperglycaemia-induced blood-brain barrier permeability, oxidative stress, and microglia activation, which compromise neuronal and glial cell integrity (62, 119). However, optimal glucose targets for infants following HI encephalopathy remain to be determined.

CONCLUSION

Hyperglycaemia is common in newborns requiring intensive care, particularly in preterm infants, and following perinatal hypoxia. The pathogenesis and clinical significance varies in each context, but hyperglycaemia is associated with increased mortality and morbidity. The limited evidence for optimal targets that impact on long term outcomes mean controversy remains regarding thresholds for intervention, and management strategies. The optimal glucose targets for infants during the acute phase of critical illness are likely to differ from those in a more stable state, when trying to achieve growth and anabolism. The first consideration in the management of hyperglycaemia must be to ascertain potentially treatable causes, followed by calculation of the GIR, to ensure it is not excessive. In term infants who are acutely unwell, restricting GIRs is likely to be more appropriate, whereas in stable extremely preterm infants where growth is considered a primary objective, one might prioritize nutritional intake with addition of insulin (4). Optimal target glucose levels remain to be determined but real-time glucose measurements and innovations in metabolomics will provide a better understanding of pathological mechanisms. This understanding, combined with real time CGM and advances in computer algorithms to provide intelligent closed loop systems, should allow a safer and more personalized approached to management in the future.

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The author confirms being the sole contributor of this work and has approved it for publication.

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