

Hyperinsulinemic Hypoglycemia Diagnosed in Childhood Can Be Monogenic

Jasmin J. Hopkins,^{1,*} Alexandra J. Childs,^{1,*} Jayne A. L. Houghton,² Thomas I. Hewat,¹ Navoda Atapattu,³ Matthew B. Johnson,¹ Kashyap A. Patel,^{1,2} Thomas W. Laver,¹ and Sarah E. Flanagan¹

¹Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter EX2 5DW, UK

²The Genomics Laboratory, Royal Devon University Healthcare NHS foundation Trust, Exeter EX2 5DW, UK

³Paediatric Endocrinology, Lady Ridgeway Hospital, Colombo 00800, Sri Lanka

Correspondence: Dr. Sarah E. Flanagan, Associate Professor in Genomic Medicine, RILD Building, University of Exeter Medical School, Barrack Road, Exeter EX2 5DW, UK. Email: S.Flanagan@exeter.ac.uk.

*These authors contributed equally to this work.

Abstract

Context: Congenital hyperinsulinism (HI) is characterized by inappropriate insulin secretion despite low blood glucose. Persistent HI is often monogenic, with the majority of cases diagnosed in infancy. Less is known about the contribution of monogenic forms of disease in those presenting in childhood.

Objective: We investigated the likelihood of finding a genetic cause in childhood-onset HI and explored potential factors leading to later age at presentation of disease.

Methods: We screened known disease-causing genes in 1848 individuals with HI, referred for genetic testing as part of routine clinical care. Individuals were classified as infancy-onset (diagnosed with HI < 12 months of age) or childhood-onset (diagnosed at age 1–16 years). We assessed clinical characteristics and the genotypes of individuals with monogenic HI diagnosed in childhood to gain insights into the later age at diagnosis of HI in these children.

Results: We identified the monogenic cause in 24% (n = 42/173) of the childhood-onset HI cohort; this was significantly lower than the proportion of genetic diagnoses in infancy-onset cases (74.5% [n = 1248/1675], $P < 0.00001$). Most (75%) individuals with genetically confirmed childhood-onset HI were diagnosed before 2.7 years, suggesting these cases represent the tail end of the normal distribution in age at diagnosis. This is supported by the finding that 81% of the variants identified in the childhood-onset cohort were detected in those diagnosed in infancy.

Conclusion: We have shown that monogenic HI is an important cause of hyperinsulinism presenting outside of infancy. Genetic testing should be considered in children with persistent hyperinsulinism, regardless of age at diagnosis.

Key Words: hyperinsulinism, hypoglycemia, genetic testing, monogenic disease, childhood

Abbreviations: HI, hyperinsulinism; IQR, interquartile range; tNGS, targeted next-generation sequencing.

Congenital hyperinsulinism (HI) is a life-threatening disorder characterized by inappropriate insulin secretion despite low blood glucose (1). Persistent HI can be monogenic, with 38% to 79% (2–5) of individuals found to have a pathogenic variant in one of the known disease-causing genes (6). The variability in these pickup rates will in part reflect how comprehensively the known genes are screened as well as the clinical features of the populations studied.

Biochemical evidence of insulin-induced hypoglycemia is a prerequisite for genetic testing; however, there are no established criteria for the age at diagnosis of HI. This is in contrast to other monogenic disorders of insulin secretion such as neonatal diabetes mellitus and maturity-onset diabetes of the young where the age at presentation of disease is a main criterion for genetic testing (7, 8).

For monogenic HI, hypoglycemia is typically diagnosed in infancy (before 12 months of age) with the most severe cases presenting within the first few days of life (9). In these individuals, disease-causing variants in the KATP channel genes, *ABCC8* and *KCNJ11*, are most common (3–5). Individuals with disease-causing variants in other commonly reported HI genes, such as *GLUD1*, often present later in infancy or, more rarely, in childhood or adulthood (4, 10).

The wide range in age at clinical presentation seen within the same monogenic forms of HI has limited its usefulness in helping to guide genetic testing of this disorder. Furthermore, no studies have systematically screened the known genes in individuals diagnosed outside of infancy, and the large, published cohorts often report limited data on the ages at presentation of HI (3, 4). Together, this makes it difficult to accurately assess how likely monogenic HI is when diagnosed outside of infancy.

In this study, we aimed to investigate the prevalence of monogenic HI in a large cohort of individuals clinically diagnosed with hyperinsulinemic hypoglycemia before 16 years of age. We then compared the pickup rates for a pathogenic variant in those diagnosed before and after 12 months of age and sought to identify genotype-phenotype relationships that could explain the variability in age at presentation of disease within the common genetic subgroups.

Methods

Participants

We studied 1848 individuals who had been referred to the Exeter Genomics Laboratory for HI genetic testing. These individuals had all received a clinical diagnosis of HI before the age of 16 years. Insulinoma had not been reported in any individuals. The cohort was divided into 2 groups; those diagnosed with HI in infancy (age < 12 months) and those diagnosed in childhood (age 1-16 years). Clinical information was provided at referral using a standardized request form. Informed consent for genetic testing was obtained from the parents or guardians with assent obtained from all individuals where appropriate. This study was approved by the North Wales Research Ethics Committee (517/WA/0327).

Molecular Genetic Analysis

Genetic testing was performed in all individuals using DNA extracted from peripheral blood leukocytes. Sanger sequencing of a single gene(s) was performed as a first line test when clinical features were highly predictive of a genetic subtype of HI. For example, *GLUD1* Sanger sequencing was performed in individuals with hyperammonemia or *ABCC8* and *KCNJ11* sequencing in individuals with diazoxide-unresponsive disease, as previously described (11–15).

Targeted next-generation sequencing (tNGS) of 11 HI genes (*ABCC8*, *CACNA1D*, *GCK*, *GLUD1*, *HADH*, *HNF1A*, *HNF4A*, *INSR*, *KCNJ11*, *PMM2*, and *TRMT10A*) was performed in all remaining individuals and those where Sanger sequencing had not identified a disease-causing variant (16). In all samples, copy number status of the targeted genes was assessed using read-depth analysis (16). The coding regions of the *KMT2D* and *KDM6A* genes were also screened in 439 individuals following the introduction of an updated tNGS gene panel to the Exeter Genomics Laboratory. When a disease-causing variant was identified, parental samples were tested when available by Sanger sequencing to establish inheritance.

Sequence data was analyzed to determine if a variant was mosaic. This was performed using the standardized allele ratio in Mutation Surveyor v3.24 software (SoftGenetics, State College, PA, USA) for samples sequenced by Sanger and allele fraction analysis for samples sequenced by tNGS.

Statistical Analysis

Birth weight Z-scores were calculated using WHO standards, accessed through the Zanthro package in STATA 16 (StataCorp, Texas, USA) (17). A Mann-Whitney test was used to compare the difference in median birth weight Z-scores. The Pearson chi-square test was used to assess the strength of categorical associations.

Results

Of the 1848 individuals studied, 1675 (90.6%) were clinically diagnosed with HI in infancy (range, birth-51.9 weeks) and 173 (9.4%) were diagnosed with HI in childhood (range, 1-15 years). For the infancy-onset cohort, the median age at diagnosis was 3 days (interquartile range [IQR], 1-21 days), with 77.9% (n = 1305/1675) diagnosed within the first month of life (Fig. 1). For the childhood-onset cohort, the median age at diagnosis was 1.75 years (IQR, 1.17-4 years).

The Monogenic Pickup Rate Was Lower in Childhood-Diagnosed HI

Disease-causing variants in 13 different genes were identified in 69.8% of the overall cohort (n = 1290/1848). The median age at diagnosis of genetically confirmed HI was 3 days (IQR, 1-14 days) with the eldest child being clinically diagnosed at 13 years (Fig. 1).

The proportion of monogenic HI was lower in the childhood-onset cohort compared to the infancy-onset cohort (24.3% [n = 42/173] vs 74.5% [n = 1248/1675], $P < 0.00001$) (Table 1 and Fig. 2). For the childhood-onset monogenic group, the median age at diagnosis was 1.71 years (IQR, 1.02-2.73 years) (Table 1). No significant differences in insulin, blood glucose or birth weight were observed between individuals with or without a genetic diagnosis in the childhood-onset group (Table 2).

GLUD1 Was the Most Common Cause of Childhood Monogenic HI

The genetic etiology was different between the 2 cohorts, with *ABCC8*-HI most common in the infancy-onset group (n = 907/1248, 73%) and *GLUD1*-HI most common in those diagnosed in childhood (n = 16/42, 38%) (Fig. 2 and Table 3). Variants in 6 genes (*KCNJ11*, *HNF4A*, *KDM6A*, *HNF1A*, *INSR*, and *CACNA1D*) were identified only in individuals diagnosed in infancy. *TRMT10A* was the only gene in which a disease-causing variant was identified exclusively in the childhood-onset cohort (Table 3). The homozygous variant was identified in a single proband who had micrognathia and a cleft palate at birth; he was diagnosed with diazoxide-responsive HI (5.2 mg/kg/day) at the age of 3 years and 4 months.

Genotype Associations Could Not Fully Explain the Variability in Ages at Diagnosis of HI

Mosaic variants were more common in the childhood-onset cohort than the infancy-onset cohort (10% vs 0.9% (n = 4/42 vs 11/1248), $P < 0.00001$, Table 1). However, 81% (n = 34/42) of variants in the childhood-onset group had been detected in individuals diagnosed with HI in infancy (Table 4), suggesting that the underlying genetics was not the sole factor influencing the age at diagnosis.

We next investigated the mode of inheritance of *ABCC8*-HI, as dominantly acting variants can cause a milder form of diazoxide-responsive disease compared with recessively inherited *ABCC8* variants (22, 23). There was no difference in the proportion of dominantly acting variants within each group (infancy cohort n = 107/907 vs childhood cohort n = 2/12, $P = 0.6$) (Table 1).

For *GLUD1*, we investigated whether the position of the variant had an impact on the age at diagnosis of HI, due to

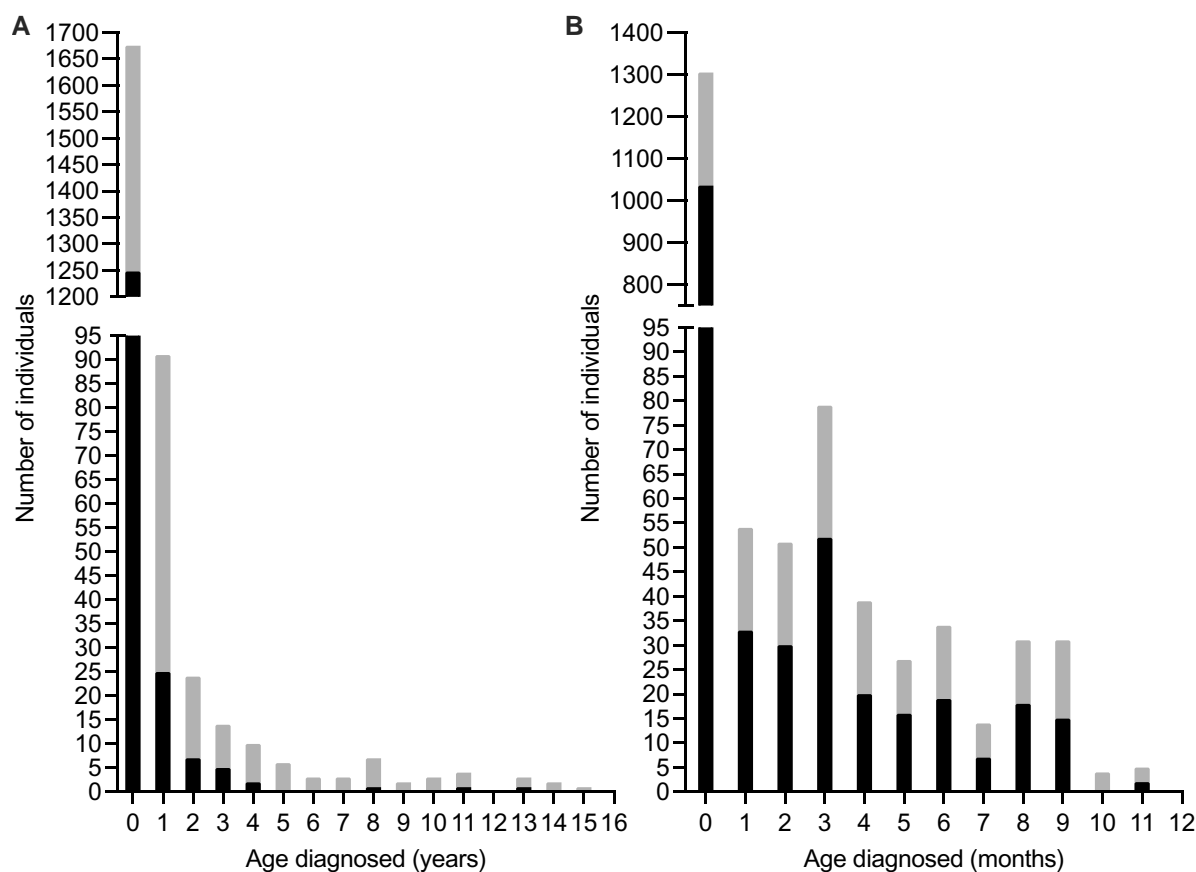


Figure 1. Distribution of age at clinical diagnosis of hyperinsulinism (HI) in probands referred for genetic testing. Gray bars indicate total number of probands, black bars indicate number of probands with a confirmed monogenic etiology. The y-axis is split to include all values. (A) Age at diagnosis of HI in years of the whole cohort (n = 1848). (B) Age at diagnosis of HI in months for those diagnosed in infancy (n = 1675). For those individuals with a genetic diagnosis, 83% (n = 1036/1248) were diagnosed within the first month of life.

Table 1. Comparison of features for those with monogenic hyperinsulinism in the infancy-onset (diagnosed < 12 months) and childhood-onset (diagnosed between 1 and 16 years) cohorts

	Infancy-onset cohort	Childhood-onset cohort	P value
Proportion monogenic	1248/1675 (74.5%)	42/173 (24.3%)	<0.00001
Median age at diagnosis (days)	3 [IQR, 1-7]	624 [IQR, 372-995]	—
Mosaic variants (%)	11 (0.9%)	4 (10%)	<0.00001
Dominant <i>ABCC8</i> (%) [n]	107 (12%) [907]	2 (17%) [12]	0.6
<i>GLUD1</i> domain catalytic:allosteric [n]	22:34 [56]	11:5 [16]	0.04*
Median birth weight Z-score [n]	1.4 [1161]	-0.3 [34]	<0.00001
Median birth weight Z-score <i>ABCC8</i> positive [n]	1.5 [843]	-0.5 [9]	0.0004
Median birth weight Z-score non- <i>ABCC8</i> -HI [n]	0.2 [136]	-0.1 [25]	0.6

*Denotes P value did not reach statistical significance after adjustment for multiple comparisons.

previous reports that the location of variants impacted the clinical presentation of disease (15, 24). We found that the *GLUD1* variants identified in the childhood-onset cohort clustered in the catalytic domain (exons 6 and 7) of the enzyme (n = 11/16, 69%), while those found in the infancy-onset cohort clustered in the allosteric domain (exons 10, 11, and 12) (n = 34/56, 61%, $P = 0.04$) (Table 1). This association was, however, not significant after correction for multiple comparisons.

Delayed Diagnosis Was Not the Main Driver of Childhood Diagnoses

As we did not detect significant variant-level associations to explain the variability in age at diagnosis, we next investigated whether a delayed diagnosis of HI was likely in those presenting in childhood. Case note review revealed that 5 individuals within the childhood-onset group (9.5%, n = 4/42) had experienced hypoglycemic episodes or seizures during infancy but

had not received a clinical diagnosis of HI at that time (Table 4). We next compared corrected birth weights, since birth weight is a surrogate marker for insulin secretion in utero, where it acts as a growth factor. The childhood-onset cohort had a significantly lower median birth weight than the infancy-onset cohort (Z-score: -0.3 vs 1.4 , $P < 0.00001$, Table 1 and Fig. 3A). These differences appeared to be driven by *ABCC8*-HI (Z: -0.5 vs 1.5 , $P = 0.0004$, Table 1 and Fig. 3B). The median birthweight in the childhood-onset cohort was similar to the population average, providing no evidence of increased insulin secretion in utero. This supports the theory that for the majority of cases, it was not undetected hypoglycemia that resulted in the later age at diagnosis.

Discussion

We identified pathogenic variants in 69.8% of individuals diagnosed with HI before the age of 16 years. This represents the minimal prevalence of monogenic HI in our cohort, since only the most commonly affected genes were screened. We further showed that HI is monogenic in a significant number of cases with childhood-onset disease, with pathogenic variants in *GLUD1* being the most common etiology.

The pickup rate was lower but still substantial in childhood-diagnosed HI, with the monogenic cause identified in 74.5% of individuals diagnosed in infancy compared to 24.3% of

individuals diagnosed in childhood. The age at diagnosis of HI in our genetically solved childhood-onset cohort ranged from 1 to 13 years, with 75% diagnosed between ages 1 and 2.7 years (Fig. 1 and Table 1). Although previous studies have separated individuals based on age at diagnosis of HI (25), this is the first study to report the prevalence of known variants within these age groups.

Some differences in the genetic subtypes of HI were observed between the infancy and childhood groups, with disease-causing variants in 6 genes identified exclusively within the infancy-onset cohort. Although it is possible that our childhood-onset cohort was underpowered to detect these rare forms of HI, we were also unable to find reports of childhood-onset cases in the literature. This suggests that variants in these genes predominantly cause early-onset HI. The only gene in which a variant was found exclusively in the childhood-onset cohort was *TRMT10A*. This represents the second reported case of a disease-causing *TRMT10A* variant in a child referred for HI genetic testing (26).

We found that mosaic variants were more common in the childhood-onset than the infancy-onset cohort ($P < 0.00001$). These variants were detected from blood samples, and we were unable to test pancreatic DNA. It seems likely that in these individuals there is a mixed population of cells within the pancreas, in which some cells do not harbor the disease-causing variant, which impacts the severity and age

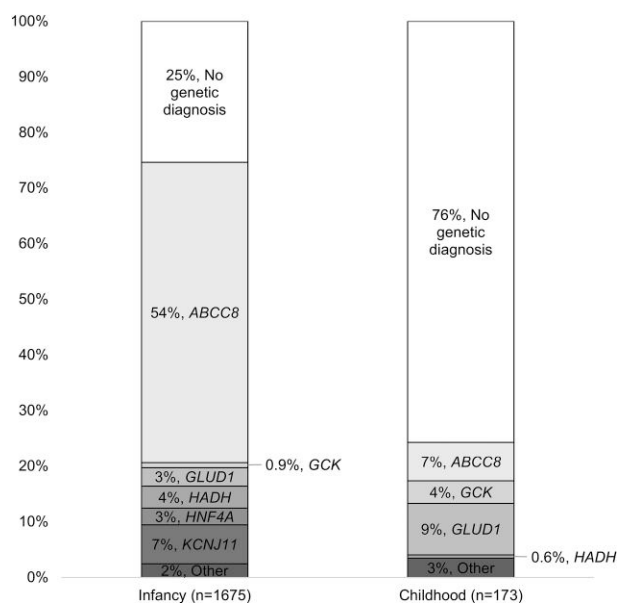


Figure 2. Comparison of genetic causes of hyperinsulinism identified in individuals diagnosed in infancy (< 12 months of age) or in childhood (between 1 and 16 years). Genes with variants in fewer than 1% of patients in the overall cohort were grouped in the “Other” category (*KMT2D*, *PMM2*, *KDM6A*, *HNF1A*, *INSR*, *CACNA1D*, and *TRMT10A*).

Table 3. Number of individuals with hyperinsulinism resulting from disease-causing variants in each gene in the infancy-onset (diagnosed < 12 months) and childhood-onset (diagnosed between 1 and 16 years) cohorts

Gene	Infancy-onset cohort n = 1248	Childhood-onset cohort n = 42	Total n = 1290
<i>ABCC8</i>	907 (4 mosaic)	12	919
<i>KCNJ11</i>	115	0	115
<i>GLUD1</i>	56 (3 mosaic)	16	72
<i>HADH</i>	66	1	67
<i>HNF4A</i>	48	0	48
<i>GCK</i>	15 (1 mosaic)	7 (3 mosaic)	22
<i>KMT2D</i>	10 (1 mosaic)	1 (mosaic)	11
<i>PMM2</i>	6	4	10
<i>KDM6A</i>	10 (2 mosaic)	0	10
<i>HNF1A</i>	8	0	8
<i>INSR</i>	5	0	5
<i>CACNA1D</i>	2	0	2
<i>TRMT10A</i>	0	1	1

The number of variants that were found to be mosaic are provided in parentheses.

Table 2. Comparison of clinical features provided at referral for individuals with hyperinsulinism diagnosed in childhood (age 1-16 years)

	Monogenic etiology confirmed n = 42	No monogenic etiology identified n = 131	P value
Insulin (pmol/L) [n]	10.6 [36]	10.7 [111]	1
Blood glucose (mmol/L) [n]	2.2 [36]	2.2 [111]	0.9
Birth weight Z-score [n]	-0.3 [34]	-0.2 [110]	0.7

P values were calculated using Mann-Whitney.

Table 4. Variants reported as causative in individuals clinically diagnosed with hyperinsulinism in childhood

Gene	Causative variant	Variant identified in an infancy diagnosis	Age at clinical diagnosis, evidence of earlier presentation and known family history
ABCC8	p.(Asp29Gly);p.(Ala1493Thr) (c.86A > G;c.4477G > A)	Yes, in reference (18); Yes, in Exeter unpublished cohort This combination of variants has not been reported	3 years
ABCC8	p.(Arg74Gln)/p.(Arg74Gln) (c.221G > A/c.221G > A)	Yes, in Exeter unpublished cohort	1.5 years, previous symptoms
ABCC8	p.(His125Gln) [†] /p.? (c.375C > G/c.3992-9G > A)	Yes, in Exeter unpublished cohort/Yes, in Exeter unpublished cohort This combination of variants has not been reported	1.5 years
ABCC8	p.(Tyr179Cys)/N (c.536A > G/N)	Yes, in reference (19)	13 years, hypoglycemia detected at age 8
ABCC8	p.(Gly228Asp)/N (c.683G > A/N)	Yes, in Exeter unpublished cohort	1 year
ABCC8	p.(Arg298Cys)/N (c.892C > T/N)	Yes, in Exeter unpublished cohort	1 year
ABCC8	p.(Trp430Ter)/N (c.1290G > A/N)	Yes, in reference (20)	1 year
ABCC8	p.(Ala1263Thr)/N (c.3787G > A/N)	Yes, in Exeter unpublished cohort	2.5 years, inherited from affected father
ABCC8	p.(Leu1431Phe) [†] /N (c.4291C > T/N)	No	1 year*, inherited from father with diabetes
ABCC8	p.(Gly1479Arg) [†] /N (c.4435G > A/N)	Yes, in Exeter unpublished cohort	1.2 years, inherited from mother with gestational diabetes
ABCC8	p.? [†] /N (c.1333-1013A > G/N)	Yes, in Exeter unpublished cohort	1.2 years
ABCC8	p.? [†] /p.? (c.1817 + 1G > C/c.1817 + 1G > C)	Yes, in Exeter unpublished cohort	1 year*
GCK	p.(Trp99Arg)/N (c.295T > C/N)	Yes, in Exeter unpublished cohort	2 years
GCK	p.(Val389Leu)/N (c.1165G > C/N)	Yes, in Exeter unpublished cohort	3.6 years*, inherited from father with poor tolerance of fasting
GCK	p.(Val389Leu)/N (c.1165G > C/N)	Yes, in Exeter unpublished cohort	3 years
GCK	p.(Val389Leu)/N (c.1165G > C/N)	Yes, in Exeter unpublished cohort	11 years
GCK	p.(Arg447Leu)/N Mosaic 30% (c.1340G > T/N)	No	1.8 years
GCK	p.(Ala454dup)/N Mosaic 17% (c.1361_1363dup/N)	Yes, in Exeter unpublished cohort	3.5 years
GCK	p.(Ala454dup)/N Mosaic 30% (c.1361_1363dup/N)	Yes, in Exeter unpublished cohort	1.8 years
GLUD1	p.(Arg221Cys)/N (c.820C > T/N)	Yes, in Exeter unpublished cohort	1.8 years
GLUD1	p.(Arg221Cys)/N (c.820C > T/N)	Yes, in Exeter unpublished cohort	2.5 years
GLUD1	p.(Arg269His)/N (c.965G > A/N)	Yes, in Exeter unpublished cohort	1.1 years
GLUD1	p.(Arg269His)/N (c.965G > A/N)	Yes, in Exeter unpublished cohort	1 year
GLUD1	p.(Arg269His)/N (c.965G > A/N)	Yes, in Exeter unpublished cohort	1.1 years
GLUD1	p.(Arg269His)/N (c.965G > A/N)	Yes, in Exeter unpublished cohort	2 years
GLUD1	p.(Arg269His)/N (c.965G > A/N)	Yes, in Exeter unpublished cohort	1.8 years

(continued)

Table 4. Continued

Gene	Causative variant	Variant identified in an infancy diagnosis	Age at clinical diagnosis, evidence of earlier presentation and known family history
<i>GLUD1</i>	p.(Arg269His)/N (c.965G > A/N)	Yes, in Exeter unpublished cohort	1.7 years
<i>GLUD1</i>	p.(Arg269His)/N (c.965G > A/N)	Yes, in Exeter unpublished cohort	4.4 years, inherited from affected mother
<i>GLUD1</i>	p.(Glu296Lys)/N (c.1045G > A/N)	No	4 years, inherited from affected mother
<i>GLUD1</i>	p.(Glu296Lys)/N (c.1045G > A/N)	No	1 year, hypoglycemic episodes possibly present from 6 months
<i>GLUD1</i>	p.(Pro436Leu)/N (c.1466C > T/N)	Yes, in Exeter unpublished cohort	1 year, seizures on day 3 of life*
<i>GLUD1</i>	p.(Ile444Met)/N (c.1491A > G/N)	No	8 years, seizures at 1 year
<i>GLUD1</i>	p.(Ser445Leu)/N (c.1493C > T/N)	Yes, in Exeter unpublished cohort	1 year, seizures within first days after birth
<i>GLUD1</i>	p.(Ser445Leu)/N (c.1493C > T/N)	Yes, in Exeter unpublished cohort	1 year
<i>GLUD1</i>	p.(Ala500Thr)/N (c.1498G > A/N)	No	2.08 years, inherited from affected father
<i>HADH</i>	p.(Lys260fs)/p.(His207Pro) (c.617delA/c.620A > C)	Yes, in Exeter unpublished cohort/No This combination of variants has not been reported	1 year
<i>KMT2D</i>	p.~/N Mosaic 20% Chr11:g.(49415449)_ (49416715)del	No	2.2 years*
<i>PMM2</i>	p.~/p.(Phe157Ser) (c.-167G > T/c.470T > C)	Yes, in Exeter unpublished cohort/No This combination of variants has not been reported	1.25 years*
<i>PMM2</i>	p.~/p.(Arg141His) (c.-167G > T/c.422G > A)	Yes, in Exeter unpublished cohort	1.5 years
<i>PMM2</i>	p.~/p.(Arg141His) (c.-167G > T/c.422G > A)	Yes, in Exeter unpublished cohort	2.8 years*
<i>PMM2</i>	p.~/p.(Asp148Asn) (c.-167G > T/c.442G > A)	Yes, in Exeter unpublished cohort/No This combination of variants has not been reported	1.1 years
<i>TRMT10A</i>	p.(Gly147fs)/p.(Gly147fs) c.440del/c.440del	No	3.3 years

Protein changes and nucleotide or genomic changes are provided for each variant. Nomenclature is given according to the following transcripts: *ABCC8* (NM_001287174.1), *GCK* (NM_000162.4), *GLUD1* (NM_005271.4), *HADH* (NM_005327.4), *KMT2D* (NM_003482.3), *PMM2* (NM_000303.2) and *TRMT10A* (NM_152292.5).

N: Denotes no pathogenic variant identified on opposite allele. All variants identified were classified as likely pathogenic or pathogenic according to ACMG guidelines (21), unless indicated by[†].

*Denotes that this individual has been reported previously in the literature. [†]Denotes a confirmed dominantly acting *ABCC8* variant.

at presentation of HI. No further significant associations between the genotype and age at presentation of disease were identified.

To assess whether a delayed clinical diagnosis could explain the variability in age at presentation of HI, we searched clinical request forms for evidence of unexplained hypoglycemia in infancy. This identified 4 individuals clinically diagnosed with HI in childhood with potential previous symptoms of hypoglycemia, including seizures. To investigate whether a delayed diagnosis was common, we studied the corrected birth weights of our cohort and found no evidence to support the presence of increased insulin secretion in utero and thus undiagnosed HI from birth in the childhood-onset cohort. In fact, we found that the birth weights of individuals diagnosed with *ABCC8*-HI in childhood were significantly lower than those diagnosed in infancy, in keeping with these being true

cases of later-onset disease rather than infancy-onset cases with a delayed diagnosis.

Taken together, the lower birth weights and the finding that 75% of individuals in the childhood-onset cohort were diagnosed before 2.7 years suggest that these monogenic cases represent the tail end of the normal distribution in age at diagnosis. This is supported by the finding that 81% of the variants identified in the childhood-onset cohort were detected in those diagnosed in infancy, suggesting that alternative genetic or environmental factors are having an impact on the age at presentation of HI in these individuals.

Our study has limitations. First, individuals were ascertained based on clinical referral. To determine the true prevalence of monogenic HI, a systematic screen is required. Second, while we screened the most common causes of HI (accounting for 69.8% of cases) we did not screen all

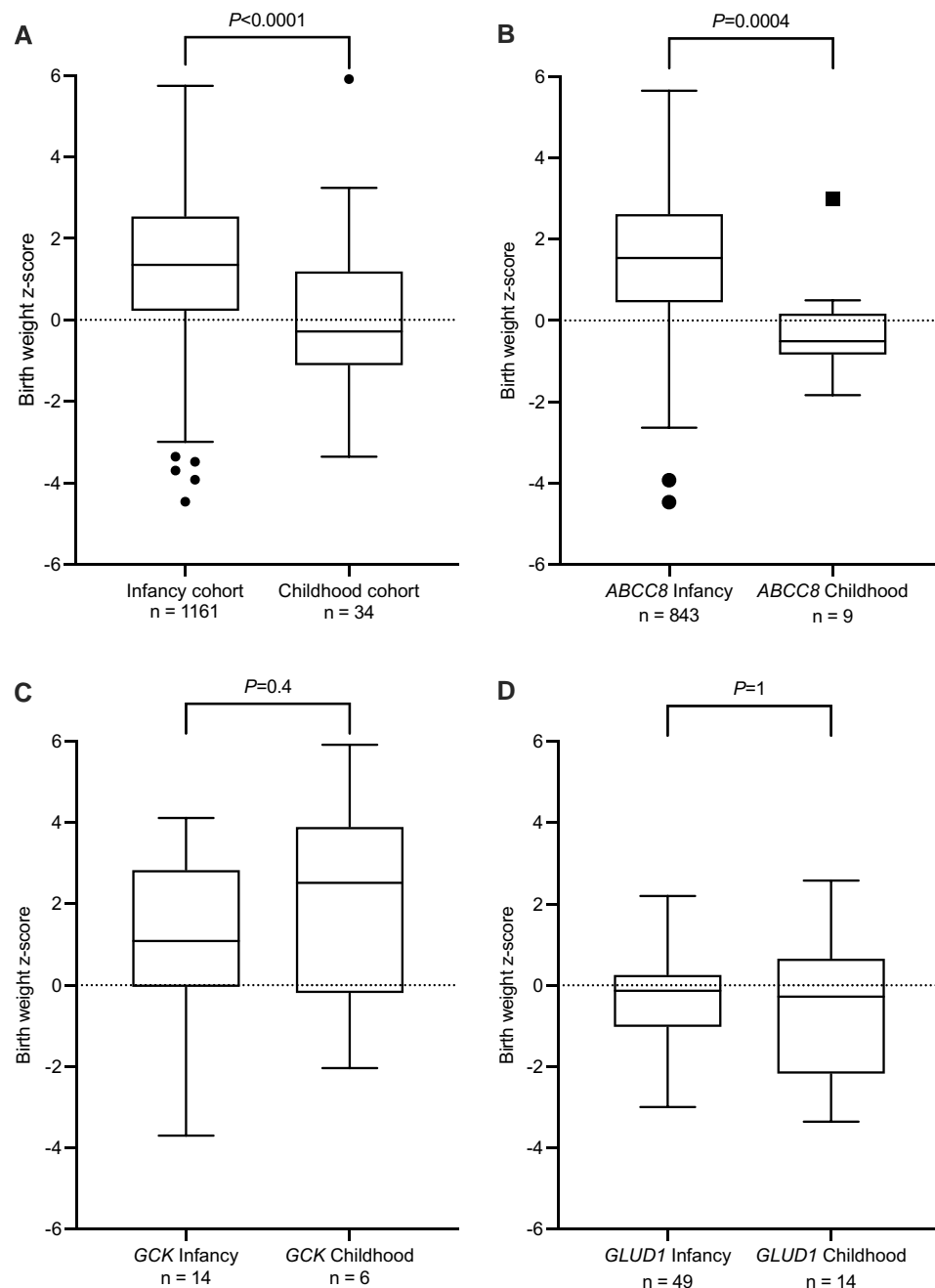


Figure 3. Comparison of birth weight z-score for infancy (< 12 months) and childhood (between 1 and 16 years) diagnosed hyperinsulinism. The middle line depicts the median birth weight Z-score, the length of the box shows the interquartile range, and the whiskers extend to a maximum of $1.5 \times$ IQR from the edge of the box, outliers are plotted individually. *P* values were calculated using Mann-Whitney. Birthweight Z-scores for all genetic causes are shown in panel (A). Data was then separated by gene where there were more than 5 individuals in both the infancy- and childhood-onset cohorts. Data for *ABCC8* is shown in panel (B) *GCK* is shown in panel (C) and *GLUD1* is shown in panel (D).

known genetic causes of HI. We may therefore have missed pathogenic variants in the genes reported to cause syndromic HI; however, this would only have a small impact since they are expected to be rare (27). Insulinomas are a well-recognized cause of HI, with the youngest reported age at diagnosis being 2 years (28); while evidence of this as an underlying cause led to exclusion, they were not systematically screened for in our cohort. We compared individuals diagnosed with HI in childhood, defined as age 1 to 16 years, to those diagnosed in infancy, younger than 12 months. While these age categories have been used in previous studies (25), alternative cutoffs

may have yielded different results. Finally, although we studied a large overall cohort, the sample size for the childhood cohort was relatively small and may not be truly representative.

In conclusion, we have shown that a significant number of individuals diagnosed with HI in childhood can have a monogenic etiology. Although the likelihood of identifying a pathogenic variant was lower for childhood-onset than infancy-onset HI, the number of children receiving a genetic diagnosis in our cohort was substantial and therefore genetic testing should be considered in all cases with persistent HI, regardless of age at diagnosis.

Acknowledgments

S.E.F. is funded by the Wellcome Trust (Grant Number 223187/Z/21/Z). T.W.L. is the recipient of a Lectureship, and M.B.J. an Independent Fellowship from the Exeter Diabetes Centre of Excellence funded by Research England's Expanding Excellence in England (E3) fund. K.A.P. has a Career Development fellowship funded by the Wellcome Trust (219606/Z/19/Z).

This research was funded in whole, or in part, by Wellcome [223187/Z/21/Z]. For the purpose of open access, the author has applied a CC BY public copyright license to any Author accepted Manuscript version arising from this submission.

Disclosures

The authors have nothing to disclose.

Data Availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

References

- Helleskov A, Melikyan M, Globa E, *et al.* Both low blood glucose and insufficient treatment confer risk of neurodevelopmental impairment in congenital hyperinsulinism: a multinational cohort study. *Front Endocrinol (Lausanne)*. 2017;8:156.
- Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S, Hussain K. Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. *Eur J Endocrinol*. 2013;168(4):557-564.
- Snider KE, Becker S, Boyajian L, *et al.* Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *J Clin Endocrinol Metab*. 2013;98(2):E355-E363.
- Männistö JME, Maria M, Raivo J, *et al.* Clinical and genetic characterization of 153 patients with persistent or transient congenital hyperinsulinism. *J Clin Endocrinol Metab*. 2020;105(4):e1686-e1694.
- El Tonbary K, Robinson P, Banerjee I, Shaikh MG. Congenital hyperinsulinism: management and outcome, a single tertiary centre experience. *Eur J Pediatr*. 2020;179(6):947-952.
- Hewat TI, Johnson MB, Flanagan SE. Congenital hyperinsulinism: current laboratory-based approaches to the genetic diagnosis of a heterogeneous disease. *Front Endocrinol (Lausanne)*. 2022;13:873254.
- Iafusco D, Stazi M, Cotichini R, *et al.* Permanent diabetes mellitus in the first year of life. *Diabetologia*. 2002;45(6):798-804.
- Ellard S, Bellanné-Chantelot C, Hattersley AT, *et al.* Best practice guidelines for the molecular genetic diagnosis of maturity-onset diabetes of the young. *Diabetologia*. 2008;51(4):546-553.
- Aynsley-Green A, Lindley KJ, Hussain K, *et al.* Practical management of hyperinsulinism in infancy. *Arch Dis Child - Fetal Neonatal Ed*. 2000;82(2):F98-F107.
- Rosenfeld E, Prakash R, Nanga R, *et al.* Characterizing the neurological phenotype of the hyperinsulinism hyperammonemia syndrome. *Orphanet J Rare Dis*. 2022;17(1):248.
- Bellanné-Chantelot C, Saint-Martin C, Ribeiro M-J, *et al.* ABCC8 and KCNJ11 molecular spectrum of 109 patients with diazoxide-unresponsive congenital hyperinsulinism. *J Med Genet*. 2010;47(11):752-759.
- Stanley CA, Lieu YK, Hsu BYL, *et al.* Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *N Engl J Med*. 1998;338(19):1352-1357.
- Proks P, Arnold AL, Bruining J, *et al.* A heterozygous activating mutation in the sulphonylurea receptor SUR1 (ABCC8) causes neonatal diabetes. *Hum Mol Genet*. 2006;15(11):1793-1800.
- Flanagan SE, Edghill EL, Gloy AL, Ellard S, Hattersley AT. Mutations in KCNJ11, which encodes Kir6.2, are a common cause of diabetes diagnosed in the first 6 months of life, with the phenotype determined by genotype. *Diabetologia*. 2006;49(6):1190-1197.
- Kapoor RR, Flanagan SE, Fulton P, *et al.* Hyperinsulinism-hyperammonemia syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. *Eur J Endocrinol*. 2009;161(5):731-735.
- Ellard S, Lango Allen H, De Franco E, *et al.* Improved genetic testing for monogenic diabetes using targeted next-generation sequencing. *Diabetologia*. 2013;56(9):1958-1963.
- Vidmar SI, Cole TJ, Pan H. Standardizing anthropometric measures in children and adolescents with functions for egen: update. *Stata J*. 2013;13(2):366-378.
- Gong C, Huang S, Su C, *et al.* Congenital hyperinsulinism in Chinese patients: 5-yr treatment outcome of 95 clinical cases with genetic analysis of 55 cases. *Pediatr Diabetes*. 2016;17(3):227-234.
- Xu Z-D, Zhang W, Liu M, *et al.* Analysis on the pathogenic genes of 60 Chinese children with congenital hyperinsulinemia. *Endocr Connect*. 2018;7(12):1251-1261.
- Park SE, Flanagan SE, Hussain K, Ellard S, Shin CH, Yang SW. Characterization of ABCC8 and KCNJ11 gene mutations and phenotypes in Korean patients with congenital hyperinsulinism. *Eur J Endocrinol*. 2011;164(6):919-926.
- Richards S, Aziz N, Bale S, *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424.
- Kapoor RR, Flanagan SE, James CT, *et al.* Hyperinsulinaemic hypoglycaemia and diabetes mellitus due to dominant ABCC8/KCNJ11 mutations. *Diabetologia*. 2011;54(10):2575-2583.
- Pinney SE, Macmullen C, Becker S, *et al.* Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant KATP channel mutations. *J Clin Invest*. 2008;118(8):2877-2886.
- Bahi-Buisson N, Roze E, Dionisi C, *et al.* Neurological aspects of hyperinsulinism-hyperammonemia syndrome. *Dev Med Child Neurol*. 2008;50(12):945-949.
- Männistö JME, Jääskeläinen J, Otonkoski T, Huopio H. Long-term outcome and treatment in persistent and transient congenital hyperinsulinism: a Finnish population-based study. *J Clin Endocrinol Metab*. 2021;106(4):1542-1551.
- Gillis D, Krishnamohan A, Yaacov B, *et al.* TRMT10A dysfunction is associated with abnormalities in glucose homeostasis, short stature and microcephaly. *J Med Genet*. 2014;51(9):581-586.
- Laver TW, Wakeling MN, Hua JHY, *et al.* Comprehensive screening shows that mutations in the known syndromic genes are rare in infants presenting with hyperinsulinaemic hypoglycaemia. *Clin Endocrinol (Oxf)*. 2018;89(5):621-627.
- Padidela R, Fiest M, Arya V, *et al.* Insulinoma in childhood: clinical, radiological, molecular and histological aspects of nine patients. *Eur J Endocrinol*. 2014;170(5):741-747.