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PHKA2 variants expand the phenotype of phosphorylase B kinase deficiency to include patients with ketotic hypoglycemia only

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Abstract

Idiopathic ketotic hypoglycemia (IKH) is a diagnosis of exclusion with glycogen storage diseases (GSDs) as a differential diagnosis. GSD IXa presents with ketotic hypoglycemia (KH), hepatomegaly, and growth retardation due to *PHKA2* variants. In our multicenter study, 12 children from eight families were diagnosed or suspected of IKH. Whole-exome sequencing or targeted next-generation sequencing panels were performed. We identified two known and three novel (likely) pathogenic *PHKA2* variants, such as p.(Pro869Arg), p.(Pro498Leu), p.(Arg2Gly), p.(Arg860Trp), and p.(Val135Leu), respectively. Erythrocyte phosphorylase kinase activity in three patients with the novel variants p.(Arg2Gly) and p.(Arg860Trp) were 15%–20% of mean normal. One patient had short stature and intermittent mildly elevated aspartate aminotransferase, but no hepatomegaly. Family testing identified two asymptomatic children and 18 adult family members with one of the *PHKA2* variants, of which 10 had KH symptoms in childhood and 8 had mild symptoms in adulthood. Our study expands the classical GSD IXa phenotype of *PHKA2* missense variants to a continuum from seemingly asymptomatic carriers, over KH-only with phosphorylase B kinase deficiency, to more or less complete classical GSD IXa. In contrast to typical IKH, which is confined to young children, KH may persist into adulthood in the KH-only phenotype of *PHKA2*.

KEYWORDS

glycogen storage disease, inborn errors of metabolism, ketotic hypoglycemia, next-generation sequencing, whole-exome sequencing

1 | INTRODUCTION

Ketotic hypoglycemia (KH) is a relatively frequent condition seen in preschool children. Rare hormonal or metabolic causes of KH include growth hormone deficiency, adrenal insufficiency, and glycogen storage disease (GSD) types 0, III, VI, and IX (Bhattacharya, 2015; Wolfsdorf & Weinstein, 2003). The most common type of KH is idiopathic ketotic hypoglycemia (IKH), also known as accelerated starvation, is loosely a diagnosis of exclusion, which is first described in 1964 (Colle & Ulstrom, 1964). IKH typically presents at the age of 1–5 years after prolonged fasting and/or fever at a male-to-female ratio of 2:1. The KH resolves after glucose administration and usually undergoes spontaneous remission at school age (Daly et al., 2003).

To establish the diagnosis of IKH, GSD is usually excluded by a normal glucagon test, normal liver parameters, and absence of hepatomegaly (Brown et al., 2015; Colle & Ulstrom, 1964). However, the glucose response to a glucagon test depends on the duration of the preceding fasting period and a normal glucagon response can be observed in all the hypoglycemia-associated subtypes GSD 0, III, VI, and IX (Bhattacharya, 2015; Kishnani et al., 2010; Wolfsdorf & Weinstein, 2003). Of note, hepatomegaly does not present usually until 6 months of age in GSD III, VI, and IX, and patients with glycogen synthesis defect due to *GYS2* DNA variations (GSD 0) do not develop hepatomegaly (Bhattacharya, 2015).

GSD IXa is caused by *PHKA2* DNA variants and accounts for 50%–75% of all types of GSD IX, which again comprises one-quarter of all GSDs (Hendrickx et al., 1995; Tsilianidis et al., 2013; van den Berg et al., 1995).

A first clue for the diagnosis of GSD IXa is KH in a family with an X-linked inheritance pattern, as *PHKA2* is located on chromosome Xp22.13. Hepatomegaly may be undiagnosed by clinical examination, especially in GSD VI and IXa (Brown et al., 2015).

We report of 12 children from five ethnic Danish and three North American families, where genetic investigations led to, or suggested, reclassification of a KH diagnosis to GSD IXa. Furthermore, the study expands the *PHKA2* phenotype to a continuum from a seemingly asymptomatic state, to KH-only, to a classical GSD IXa phenotype with hepatomegaly of varying degrees, delay in growth, and sometimes KH varies from occasional after prolonged fasting or illness to recurrent after an overnight fast (Kishnani et al., 2019).

2 | MATERIALS AND METHODS

2.1 | Editorial policies and ethical considerations

Ethical permission to perform whole-exome sequencing (WES) in Families A and B was obtained from The Regional Ethic Committee of Southern Denmark (J.no. S-VF-20040235).

The study was approved by the Danish Patient Safety Authority (reference no: 3-3013-2484/1) and The Danish Data Protection Agency (reference no: 18/14746). Participants signed informed consent for the WES analysis. The variants found were reported to the ClinVar database (Database, 2018) based on GenBank accession number NM_000292.2.

2.2 | Materials and methods

In this multicenter study, patients diagnosed with IKH and having a history of known or suspected familial hypoglycemia were identified at Hans Christian Andersen Children's Hospital, Odense University Hospital, Denmark; Stollery Children's Hospital, University of Alberta, Canada; Alberta Children's Hospital, University of Calgary, Canada; or Children's Hospital of Philadelphia, PA, USA. Clinical data from probands and family members were obtained retrospectively from the hospital charts. WES was performed in Families A and B as described elsewhere (Alhaidan et al., 2020). Probands of Families C, F, and H underwent targeted next-generation sequencing (NGS) of a 29-, or 50-, gene GSD or hypoglycemia panel including deletion/duplication analysis (Blueprint Genetics, Espoo, Finland). In Families D and G, targeted NGS of a 22-gene monogenic hypoglycemia panel was performed at Prevention Genetics (Marshfield, WI, USA). Proband E underwent direct sequencing of *GYS2*, *PYGL*, and *PHKA2*, and tests for a congenital disorder of glycosylation (CDG) and mitochondrial DNA (mtDNA).

The specific gene content of each panel is listed in Supplementary Table 1.

A clinical geneticist performed expanded family investigations in Families A–F. *PHKA2* variants were reported based on GenBank accession numbers NM_000292.2 and NP_000283.1. For a novel variant, pathogenicity was scored by in silico analyses, including Combined Annotation-Dependent Depletion (Kircher et al., 2014). The pathogenicity was determined according to the ACMG guidelines (Richards et al., 2015). Chromosome microarray was performed by CytoScan™ HD, Affymetrix. Clinical follow-up was performed after the genetic investigations.

Glucagon stimulation tests were performed according to Soltész et al. (Soltész et al., 2003). Elastography, an ultrasound-based imaging technique, was performed using Fibroscan®. By elastography, both steatosis and fibrosis can be assessed and expressed as the controlled attenuation parameter (CAP) and liver stiffness, respectively (Sasso et al., 2010).

3 | RESULTS

3.1 | Family A

3.1.1 | Proband and sisters

In this Danish family, the female proband (III:1, Figure 1a) had onset of KH at 17 months of age after an overnight fast and excess physical activity the day before. On repeated attacks, KH was documented (Table 1). Whipple's triad (hypoglycemia symptoms; low plasma glucose; relief of symptoms after glucose administration) was positive.

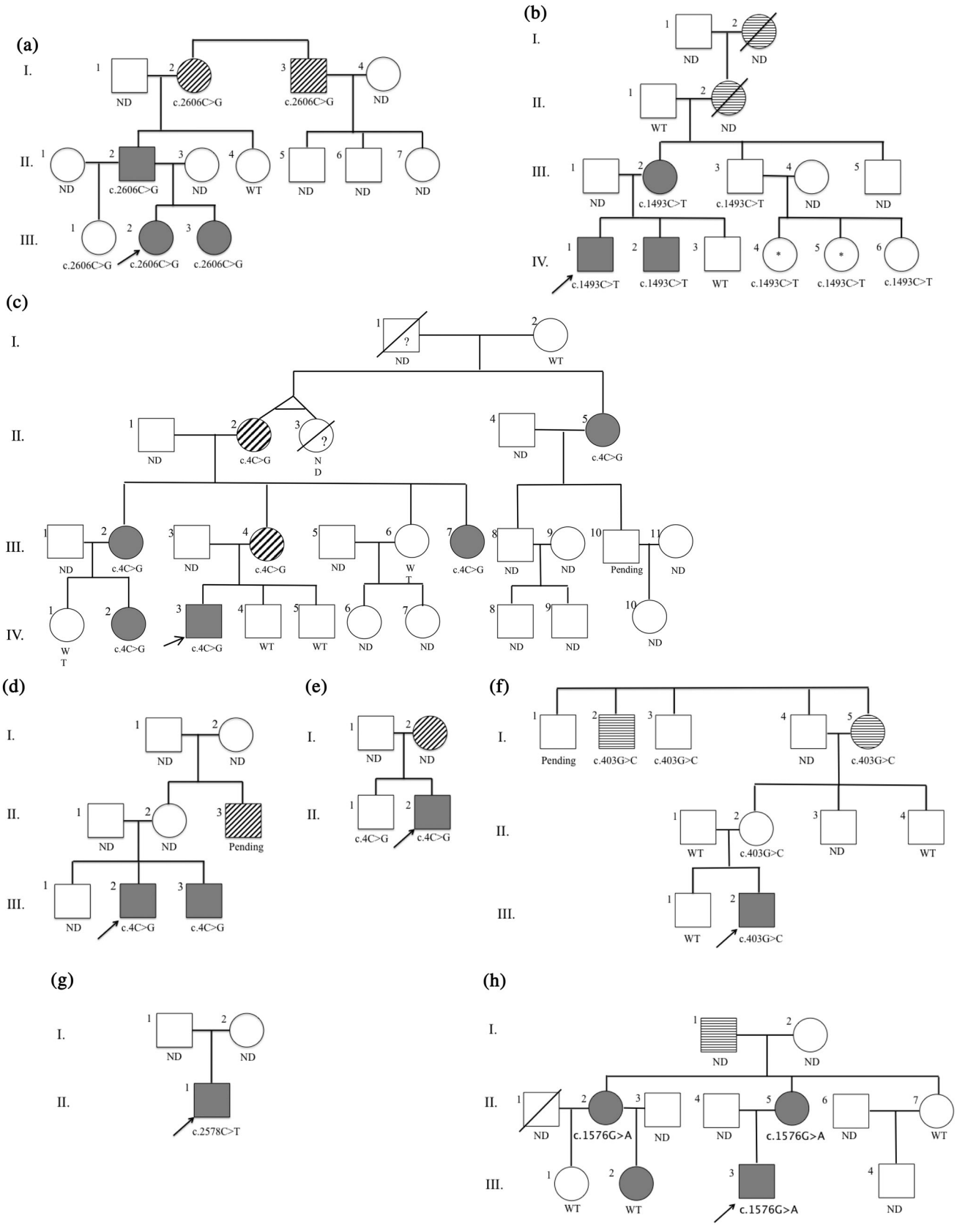

FIGURE 1 Pedigrees of the eight families. ND, no data; WT, wild type. Arrow: proband. Gray: hypoglycemia symptoms in childhood to present age. Diagonal hatch: hypoglycemia symptoms in childhood. Horizontal hatch: Type 2 diabetes. White: no ketotic hypoglycemia symptoms. * indicates repeat febrile convulsions in infancy, no glucose data

TABLE 1 Clinical and paraclinical characteristics of the probands and young family members with ketotic hypoglycemia and PHKA2 variants in eight families

Patient	*PHKA2* DNA variant	Sex (male/female)	Present age (years)	Clinical onset (month/years)	Symptoms	Lowest blood glucose (mmol/L)	Highest ketones[a]	Lowest HbA1c (ref. 31–44) (mmol/mol)	Hepatomegaly (ultrasound or clinical)	ALT and AST (ref. 10–45) (U/L)	Height ≤ 2 SD	Last KH attacks	Clinical status at last follow-up
Family A	c.2606C>G, p.(Pro869Arg)												
III:1	C>G	F	13 y	–	Reportedly asymptomatic	ND	ND	36	ND	<45	No	Asymptomatic	Normal fasting PG and HbA1c
III:2 (P)	C>G	F	10 y	17 m	Lethargy, vomiting, sweating, shivering	2.5	5.1	33	No (u + c)	<45	No	8½ y; PG 2.8 mmol/L, BK 3.5 mmol/L	Mild symptoms during sport, no treatment
III:3	C>G	F	8 y	3 y	Frequent acetone odor and nausea in mornings	2.8	ND	30	ND	<45	No	Ongoing	Frequent acetone odor and nausea in mornings, dietary treatment initiated
Family B	c.1493C>T, p.(Pro498Leu)												
IV:1 (P)	C>T	M	19 y	19 m	Frequent episodes with morning shivering, uneasiness, pale, hunger or nausea, drowsiness, relief after food	1.9	+2 ketonuria	29	No (u + c)[b]	<45	No	12 y	Asymptomatic, no treatment
IV:2	C>T	M	15 y	17 m	Frequent episodes with morning shivering, uneasiness, pale, tachycardia, nausea, vomiting, drowsiness. Relief after food	2.1	+4 ketonuria; 8 mmol/L	32	No (u + c)	<45	No	Ongoing	KH episodes every other month
Family C	c.4C>G, p.(Arg2Gly)												
IV:2	C>G	F	11 y	Recognized at 6 y	Afebrile fasting episodes of vomiting, shakiness, uneasiness, drowsiness, relief after food	2.2	1.6 mmol/L	33	No (c)	<45	No	9½ y	Asymptomatic, normal fasting PG and HbA1c, no treatment

TABLE 1 (Continued)

Patient	*PHKA2* DNA variant	Sex (male/female)	Present age (years)	Clinical onset (month/years)	Symptoms	Lowest blood glucose (mmol/L)	Highest ketones[a]	Lowest HbA1c (ref. 31–44) (mmol/mol)	Hepatomegaly (ultrasound or clinical)	ALT and AST (ref. 10–45) (U/L)	Height ≤ 2 SD	Last KH attacks	Clinical status at last follow-up
IV:3 (P)	C>G	M	7½ y	8 mo	Afebrile fasting episodes of vomiting, nausea, shakiness, uneasiness, pallor, sweating, drowsiness, hypotonia. Shortly unconscious during febrile episodes	1.8	3.1 mmol/L	33	No (u + c)	<45	No	7½ y	7½ y; unchanged afebrile KH attacks 1–3 times per month. Preventive dietary long carbohydrates and uncooked cornstarch, CGM
Family D	c.4C>G p.(Arg2Gly)												
III:2 (P)	C>G	M	5 y	16 m. Hypoglycemic seizure; hypoglycemia (rebound?) after emergency i.m. glucagon	Frequent early morning ketotic hypoglycemia with hunger, altered level of consciousness, prompt relief after food.	2.1	1.6	ND	No (u)	<45	No	Ongoing	Fasting avoidance, nocturnal cornstarch
III:3	C>G	M	3 y	9 m. Unresponsive in his crib at morning during sleep-training	Frequent early morning ketotic hypoglycemia with hunger, altered level of consciousness, prompt relief after food.	1.3	1.5	ND	No (u)	<45	No	Ongoing	Fasting avoidance, nocturnal cornstarch
Family E	c.4C>G p.(Arg2Gly)												
II:1	C>G	M	Adolescence	–	Reportedly asymptomatic	ND	ND	ND	ND	ND	No	Asymptomatic	Asymptomatic, no treatment
II:2 (P)	C>G	M	Adolescence	Recognized age 2.2 y	Trembling and sweating after an overnight fast	1.1	4.2	ND	No (u + c)	AST occasionally mildly elevated	< 5th percentile	13 y	Fast overnight, uncooked corn starch at bedtime
Family F	c.403G>C, p.(Val135Leu)												
III:2 (P)	G>C	M	6 y	24 m	Mostly morning afebrile attacks of hypotonia, sweating, drowsiness, shakiness, vomiting, relief after food	2.9	4.1	32	Marginal (u)[c]	<45	No	Ongoing	Fewer episodes after dietary treatment, CGM guided. Cornstarch not needed.

(Continues)

TABLE 1 (Continued)

Patient	*PHKA2* DNA variant	Sex (male/female)	Present age (years)	Clinical onset (month/years)	Symptoms	Lowest blood glucose (mmol/L)	Highest ketones[a]	Lowest HbA1c (ref. 31–44) (mmol/mol)	Hepatomegaly (ultrasound or clinical)	ALT and AST (ref. 10–45) (U/L)	Height ≤ 2 SD	Last KH attacks	Clinical status at last follow-up
Family G	c.2578C>T, p.(Arg860Trp)												
II:1 (P)	C>T	M	5 y	3.5 y	Frequent early morning night-terror-like agitated episodes. Agitated screaming / premature waking, relieved by food. Occasionally with vomiting	2.0	ND	54	No (u)	<45	No	Ongoing	High-protein diet, fasting avoidance, qHS cornstarch
Family H	c.1576G>A, p.(Asp526Asn)												
III:2	WT	F	4 y	20 m	Morning uneasiness, shakiness, sweating, drowsiness, relief after food	2.3	4.1	31	No (u + c)	<45	No	Ongoing	No episodes after treatment with uncooked cornstarch and diazoxide 2.5–3 mg/kg/d
III:3 (P)	G>A	M	9 y	Recognized at 6½ y	Afebrile, mostly morning episodes of vomiting, nausea, shakiness, uneasiness, sweating, drowsiness, hypotonia, relief after food	2.3	3.2	29	No (u + C)	<45	No	Ongoing	Fewer episodes after dietary treatment, nocturnal cornstarch

Note: All probands had an initial diagnosis of IKH after exclusion of hypoglycemia due to liver, metabolic, and hormonal disease, including growth hormone and adrenal insufficiency. Intramuscular glucagon test was normal in the probands A–D and G. Muscle and skin biopsy investigations were normal in the Family B patient IV:2; normal muscle biopsy in Family D patient III:1.

Abbreviations: C, clinical; CGM, continuous glucose monitoring; F, female; M, male; ND, No diagnosis; P, proband; PG, P-glucose; U, ultrasound.

[a]Plasma betahydroxybutyrate (ref. <0.6 mmol/L9, or urine acetoacetate stix (ref. +0).

[b]Ultrasound showed hyperechogenicity.

[c]Also marginal hyperechogenicity 3½ y old, repeat ultrasound normal age 5½ y.

The girl had no additional abnormal clinical or paraclinical features, and she was diagnosed with IKH. Liver biochemical parameters are given in Supplemental Table 2. She was treated with uncooked cornstarch, a diet with slow release carbohydrate before night, and sugar-rich drinks and meals at attacks, with good effect. On follow-up until 10 years of age, she had occasional mild hypoglycemia symptoms after sports activity only.

A WES analysis identified a rare, heterozygous, paternal, and predicted disease-causing variant in *PHKA2*, c.2606C>G, p.(Pro869Arg), confirmed by Sanger sequencing. The variant was previously reported with low erythrocyte phosphorylase kinase (PhK) activity (17%–25% of normal mean) in GSD IXa (Beauchamp et al., 2007) and was considered pathogenic (Table 2).

The c.2606C>G variant was also identified in two sisters of the proband (III:1, III:3). The 13-year-old sister reportedly never had KH symptoms, but she was advised to check glucose and ketones on eventual attacks. Retrospectively, the younger sister identified KH symptoms since 3 years of age and subsequently monitoring revealed glucose down to 2.8 mmol/L. Ketones were never obtained.

3.1.2 | Family investigations

The *PHKA2* variant c.2606C>G was identified in three adult family members (I:2, I:3, and II:2) consistent with X-linked inheritance (Table 3). All had KH symptoms in childhood, but their fasting glucose and glycated hemoglobin (HbA1c) were normal at present. In adulthood, the proband's father had however occasional KH symptoms but normal liver investigations. The father's sister (II:3) had a borderline fasting glucose of 3.1 mmol/L but was wild type.

3.2 | Family B

3.2.1 | The proband and his brother

Two Danish brothers (proband IV:1 and IV:2, Figure 1b) presented with severe episodes of KH from 19 and 17 months' age, respectively. Whipple's triad was positive. Both brothers had occasionally elevated plasma pyruvate between 94 and 294 (ref. 30–80) mmol/L, lactate up to 3.9 (ref. 0.5–2.1) mmol/L; free fatty acids up to 2.25 (ref. 0.1–0.5) mmol/L upon hypoglycemia. Patient IV:2 had severe hearing loss diagnosed at 10 months of age, treated with a cochlear implant. No genetic cause to congenital neurosensory deafness was identified with a hearing loss NGS panels or chromosomal microarray, and none of the family members suffered from hearing loss. No additional clinical or paraclinical features were identified. Comprehensive hormonal and metabolic investigations in the boys failed to identify a cause to the presumed identical hypoglycemia disease, which led to a diagnosis of IKH.

The brothers were treated with frequent meals and uncooked cornstarch before bedtime. In both brothers, WES identified a rare, hemizygous, predicted disease-causing variant in *PHKA2*, c.1493C>T, p.(Pro498Leu), previously reported in GSD IXa (Beauchamp et al., 2007) and considered likely pathogenic (Table 2).

On follow-up, up to 19 years of age, the eldest brother (IV:1) had a normal-sized liver by ultrasound, normal biochemical liver profile, and normal plasma pyruvate and lactate. Transient subclinical hypothyroidism was noted. He had been asymptomatic for 7 years and without treatment. By the age of 18 years, liver ultrasound showed hyperechogenicity but a normal-size liver. Liver biopsy showed discrete fibrosis, without steatosis. Unfortunately, glycogen content could not be evaluated given the lack of fresh frozen tissue. Elastography showed a CAP medium value of 277 (ref. <250) dB/m. The stiffness of the liver was normal, 4.3 (ref. <6) kPa.

On follow-up of the younger brother (IV:2) up to 16 years age, ALT (alanine aminotransferase) was marginally increased, Supplemental Table 2.

3.2.2 | Family investigations

The *PHKA2* variant c.1493C>T was found in the mother (III:2), her eldest brother (III:3), and his three daughters (IV4-6), consistent with X-linked inheritance.

The mother (III:2) reported repeated episodes of mild hypoglycemia since childhood, self-treated with frequent meals and snacks, while the other family members were asymptomatic.

One daughter (IV:5) had slightly elevated ALT and gamma-glutamyl transferase. Her ultrasound of the liver revealed a normal-sized but hyperechogenic liver. Besides this finding, all had normal fasting glucose and HbA1c, ALT, and hepatic ultrasound.

The deceased maternal grandmother (II:1) and great-grandmother (I:1) were both obese with Type 2 diabetes (T2D) in adulthood.

3.3 | Family C

3.3.1 | The proband and his cousin

In the Danish Family C, the male proband (IV:3, Figure 1c) diagnosed at 8 months of age presented with symptomatic recurrent morning fasting hypoglycemia with glucose down to 1.8 mmol/L; ketones 3.1 mmol/L. In the investigation phase, an arginine growth hormone stimulation test was discontinued due to hypoglycemia and prompted growth hormone treatment at the age of 2.5–3 years with no effect on hypoglycemia. Other pituitary hormones and metabolic investigations were normal. Given a diagnosis of IKH, dietary treatment with long-acting carbohydrates and uncooked cornstarch prevented severe hypoglycemic attacks; however, episodes of morning fasting vomiting persisted.

By a 29-gene NGS panel, a novel, hemizygous, and rare *PHKA2* missense variant was identified, c.4C>G, p.(Arg2Gly), which was also found in Families D and E and was considered pathogenic (Table 2).

On follow-up, until age 7.5 years, the proband still experienced hypoglycemia episodes in the morning with fatigue and vomiting.

TABLE 2 Predictors and interpretation of *PHKA2* DNA changes in the eight families

Family number	A	B	C, D, E	F	G	H
Genomic (GRCh38-v1.6)	g.18906806	g.18925744	g.18983929	g.18951155	g.18907037	g.18924519
DNA change	c.2606C>G	c.1493C>T	c.4C>G	c.403G>C	c.2578C>T	c.1576G>A
Nucleotide change	p.(Pro869Arg)	p.(Pro498Leu)	p.(Arg2Gly)	p.(Val135Leu)	p.(Arg860Trp)	p.(Asp526Asn)
GnomAD (%)	0.00054	0.0071	0.0044	0.0	0.022	0.0039
dbSNP (%)	−	0.03	−	−	−	−
HGMD (+/−)	+	+	−	−	−	−
SIFT	Deleterious (score: 0.02)	Deleterious (score: 0.01)	Deleterious (score: 0.03)	Tolerated (score: 0.08)	Deleterious (score: 0.01)	Deleterious (score: 0)
PolyPhen2	Probably damaging (score: 0.999)	Possible damaging (score: 0.903)	Benign (score: 0.024)	Benign (score: 0.013)	Probably damaging (score: 0.995)	Probably damaging (score: 0.981)
MuPro	Decrease stability (delta $G = -0.57601864$)	Decrease stability (delta $G = -0.1972622$)	Decrease stability (delta $G = -1.487982$)	Decrease stability (delta $G = -0.72841813$)	Decrease stability (delta $G = -0.2265332$)	Decrease stability (delta $G = -0.9985107$)
SNAP	Neutral (−74)	Effekt (56)	Neutral (−6)	Neutral (−85)	Neutral (−96)	Neutral (−31)
SNPs&Go	Neutral (0.085)	Disease (0.553)	Neutral (0.344)	Neutral (0.047)	Neutral (0.330)	Disease (0,714)
Mutationtaster	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)	Disease causing (prob: 1)
PhD-SNP	Neutral (0.0.452)	Disease (0.816)	Neutral (0.221)	Neutral (0.167)	Neutral (0.318)	Disease (0.606)
PANTHER	Neutral (0.227)	Disease (0.623)	Disease (0.827)	Neutral (0.101)	Disease (0.930)	Disease (0,606)
CADD (PHRED)	27.1	26.6	23.6	18.67	29.0	27.3
Interpretation by ACMG guidelines	Pathogenic (PS3, PS4 moderate, PM2, PP2, PP3, PP4)	Likely pathogenic (PS4 moderate, PM1, PM2, PP1, PP2, PP3, PP4)	Pathogenic (PS3, PS4 moderate, PM2, PP2, PP3, PP4)	Likely pathogenic (PM1, PM2, PP2, PP3, PP4)	Likely pathogenic (PS3, PM6, PP2, PP3, PP4)	Variant of uncertain significance (PM2, PP2, PP3, PP4)

Note: DNA and nucleotide change according to NM_000292.3 and NP_000283.1.

TABLE 3 Clinical and paraclinical characteristics of selected family members in the eight families

		Sex	Childhood history		Actual data							
Patient	*PHKA2* DNA variant	(male/female)		Age (years)	KH symptoms	Fasting p-glucose (ref. 4–6) (mmol/L)	HbA1c (ref. 31–44) (mmol/mol)	Ketosis[a] (ref. <0.6 mmol/L)	Hepatomegaly (ultrasound or clinical)	Short stature ≤2 SD	Treatment	Comments
Family A	c.2606C>G, p.(Pro869Arg)											
I:2	C>G	F	Recurrent nausea, vomiting, sweating, uneasiness	66	No	5.0	36	ND	ND	No	Dietary	
I:3	C>G	M	At least from schoolage: frequent morning nausea, uneasiness, relief after sugar-rich meal. Remission around 16 years' age	59	No	6.1	32	ND	ND	No	None	
II:2	C>G	M	Since at least from 7 years, recurrent morning nausea, vomiting, sweating, shivering, uneasiness, relief after food	40	Less frequent morning nausea and uneasiness. After large meals sweating, shivering, uneasiness, vomiting	5.3	30	ND	No (u)	No	Dietary	
Family B	c.1493C>T, p.(Pro498Leu)											
III:2	C>T	F	Occasional morning attacks with shivering, seating, uneasiness	46	Rare episodes with tremor, uneasiness, sweating	5.4	34	ND	No (u)	No	Frequent meals	
III:3	C>T	M	Asymptomatic	44	Asymptomatic	6.0	37	ND	No (u)	No	None	
IV:4	C>T	F	Repeat febrile convulsions in infancy[b]	21	Asymptomatic	5.3	31	ND	No (u)	No	None	

(Continues)

TABLE 3 (Continued)

Patient	*PHKA2* DNA variant	Sex (male/female)	Childhood history	Age (years)	Actual data: KH symptoms	Fasting p-glucose (ref. 4–6) (mmol/L)	HbA1c (ref. 31–44) (mmol/mol)	Ketosis[a] (ref. <0.6 mmol/L)	Hepatomegaly (ultrasound or clinical)	Short stature ≤2 SD	Treatment	Comments
IV:5	C>T	F	Repeat febrile convulsions in infancy[b]	18	Asymptomatic	5.2	33	ND	No (u)[c]	No	None	
IV:6	C>T	F	Asymptomatic	13	Asymptomatic	ND	34	ND	ND	No	None	
Family C	c.4C>G, p.(Arg2Gly)											
II:2	C>G	F	Nausea, poor eating, low weight, hospitalized for attacks of unknown nature	62	Episodes with morning nausea, uneasiness, pallor, sweating, shakiness, relief after food	ND	37	ND	ND	No	Dietary	
II:3	ND	F	Died 3 months old, no further data									
II:5	C>G	F	Mild uneasiness when hunger, relief after food	57	Mild uneasiness when hunger, relief after food	5.6	37	ND	ND	No	None	
III:2	C>G	F	Frequent episodes with uneasiness, pallor, vomiting, drowsiness, shakiness, relief after food up to 12 y. age	39	Episodes with uneasiness, shakiness, pallor, vomiting; relief after food	4.3	33	No	No (u)	No	None	
III:4	C>G	F	Episodes as teenager with uneasiness, shakiness, floppyness, pallor, sweating, relief after food	38	Fasting for OGTT during pregnancy led to severe uneasiness, shakiness, nausea, dizziness	4.8	36	ND	ND	No	None	
III:7	C>G	F	Episodes with morning nausea, uneasiness, pallor, sweating, shakiness, relief after food	22	Episodes with morning nausea, uneasiness, pallor, sweating, shakiness, relief after food	ND	ND	ND	ND	No	None	Normal X-chromosome distribution

TABLE 3 (Continued)

Patient	*PHKA2* DNA variant	Sex (male/female)	Childhood history	Age (years)	Actual data: KH symptoms	Fasting p-glucose (ref. 4–6) (mmol/L)	HbA1c (ref. 31–44) (mmol/mol)	Ketosis[a] (ref. <0.6 mmol/L)	Hepatomegaly (ultrasound or clinical)	Short stature ≤2 SD	Treatment	Comments
Family D	c.4C>G p.(Arg2Gly)											
II:3	ND (pending)	M	Vague history of recurrent hypoglycemia in childhood, now less prominent	33	Weakness and/or mental fuzziness relieved by food	4.3	ND	ND	ND	No	Symptomatic only	
Family E	c.4C>G p.(Arg2Gly)											
I:2	ND	F	Symptoms after prolonged fasting	Adult	ND	ND	ND	ND	ND	152.4 cm (5th percentile)	None	
Family F	c.403G>C, p.(Val135Leu)											
I:2	G>C	M	ND	76	No	9.8	56	ND	ND	No	Diet for T2D	T2D
I:3	G>C	M	ND	73	No	5.8	38	ND	ND	No	None	
I:5	G>C	F	No reported symptoms	70	No	9.7	56	ND	Yes[c]	No	Diet for T2D	Obesity, T2D
II:2	G>C	F	No reported symptoms	32	Asymptomatic	5.1	30	ND	No (u)	No	None	
Family G	No data on parents or other family members											
Family H	c.1576G>A, p.(Asp526Asn)											
I:1	ND	M	ND		Repeat shakiness, convulsions, relief after food	ND	ND	ND	ND	ND	Dietary	T2D, gastric bypass
II:2	ND	F	Frequent episodes, fasting and postprandial, with severe uneasiness, sweating, shakiness, irritability, relief after food, recognized since 15 y	38	Frequent episodes, fasting and postprandial, with severe uneasiness, sweating, shakiness, irritability, relief after food. Both before and after gastric bypass in 2011	3.1	31–42[d]	ND	ND	No	Dietary	Severe obesity, BMI 48.9 kg/m^2, PCOS

(Continues)

TABLE 3 (Continued)

Patient	*PHKA2* DNA variant	Sex (male/female)	Childhood history	Age (years)	Actual data KH symptoms	Fasting p-glucose (ref. 4–6) (mmol/L)	HbA1c (ref. 31–44) (mmol/mol)	Ketosis[a] (ref. <0.6 mmol/L)	Hepatomegaly (ultrasound or clinical)	Short stature ≤2 SD	Treatment	Comments
II:5	G>A	F	Frequent episodes with severe nausea, uneasiness, sweating, shakiness, irritability, relief after food, recognized since 13 y	31	Frequent episodes with severe nausea, uneasiness, sweating, shakiness, irritability, relief after food.	6.7	33–47	ND	ND	No	Dietary	Severe obesity, BMI 55.6 kg/m^2, PCOS
II:7	ND	F										T2D, PCOS

[a]Plasma betahydroxybutyrate >1.0 mmol/L.
[b]No blood glucose values obtained.
[c]Hyperechogenicity of the liver.
[d]After gastric bypass.

The female cousin (IV:2) had hypoglycemia symptoms since 6 years of age and ketosis was verified at glucose 2.2 mmol/L by the age of 7 years, 8 months. On follow-up until 11 years old, she was asymptomatic since 9.5 years of age.

3.3.2 | Family investigations

The novel *PHKA2* missense variant c.4C>G segregated in the family in an X-linked pattern affecting five other family members (Figure 1c). All had present or former KH symptoms; however, with present normal glucose and liver investigations, except III:7 with marginally elevated ALT (Table 3 and Supplemental Table 2).

Pronounced hypoglycemia symptoms in the 22-year-old female (III:7) prompted leukocyte investigations for skewed X-chromosome inactivation. The X-chromosome distribution was, however, normal. Of special note, the grandmother's monozygotic twin (II:3) died 3 months old of unknown reason.

3.4 | Family D

3.4.1 | Two brothers

In this Canadian family, the proband (III:2, Figure 1d) presented at age 16 months with an early-morning seizure and hypoglycemia (2.2 mmol/L). He refused his evening meal the night prior to the presentation; Whipple's triad was positive. Repeat symptomatic KH episodes generally occurred between midnight and breakfast, more likely at times of intercurrent illness, and were readily responsive to enteral or parenteral carbohydrates. Despite frequent feeds every 3 h while awake, cornstarch (~0.5 g/kg/day) every 5–6 h overnight, he experienced five to six episodes per year of hypoglycemia <2 mmol/L, generally managed at home with oral carbohydrates.

His brother (III-3) presented at 9 months of age with early morning hypoglycemia and was found unresponsive in his crib the morning after his parents changed his overnight feeding schedule (from a night bottle to a single early morning bottle). Point-of-care testing (POCT) showed a glucose concentration of 1.3 mmol/L and blood ketones of 3.5 mmol/L. Whipple's triad was met. He experienced multiple frequent episodes of nocturnal and early-morning hypoglycemia, despite a parent-managed regimen similar to that of his brother but a somewhat higher nocturnal cornstarch dose, ~1.1 g/kg/dose, q5h.

Both III-2 and III-3 had otherwise normal extensive endocrine and metabolic investigations including normal liver size and echotexture by ultrasound, fasting blood lactate, urate, triglycerides (except one borderline-increased concentration, 1.78 mmol/L; R.I. <1.50) in III-3, plasma amino acids, acylcarnitine profile, and urine organic acids. Follow-up until 5 and 3 years, respectively, revealed ongoing, repeat episodes of KH.

An NGS hypoglycemia panel in III-3 showed a novel hemizygous *PHKA2* variant, c.4C>G, p.(Arg2Gly) as identified in the Danish Family C. PhK activity in both brothers blood was 20% of mean-normal

(0.2 μmol/min/gHgb), that is, twice the usual level in persons with classical GSD IXa (≤0.1 μmol/min/gHgb). The testing laboratory had observed the p.(Arg2Gly) variant in three other hemizygous boys referred for hypoglycemia.

3.4.2 | Family investigations

On review of the family history, the patient's mother presented no history of hypoglycemia or compatible symptoms. A maternal uncle (II-3) offered a similar history of unexplained episodes of hypoglycemia in childhood; his symptoms have lessened over time such that he no longer monitors his blood glucose levels. In adulthood, he still had frequent morning time weakness and/or mental fogginess relieved by eating.

3.5 | Family E

3.5.1 | The proband and his brother

The American male proband (II:2, Figure 1e) was referred to a hypoglycemic specialist center at age 2.5 years. He had home-monitored plasma glucose down to 1.1–1.7 mmol/L. Two fasting tests showed glucose of 2.3–2.5 mmol/L after 8 h with beta-hydroxybutyrate (BOHB) 4.1–4.2 (ref. <0.6) mmol/L. Routine hormonal and metabolic screening showed starvation patterns and a growth hormone concentration during hypoglycemia of 0.651 ng/ml. His height and weight were below the fifth percentile despite a normal mid-parental height of 170.3 cm, but growth hormone stimulation tests were normal. A trial of growth hormone treatment was stopped as it failed to prevent hypoglycemia. After uncooked cornstarch (1.85 mg/kg) at bedtime age 3½ years, 19.5 h fasting resulted in a glucose concentration of 2.6 mmol/L with BOHB of 3.3 mmol/L and free fatty acids of 5.8 (ref. 0.1–0.5) mmol/L. A subsequent glucagon dose of 1 mg did not increase his plasma glucose.

Liver investigations were normal apart from an occasional mild elevation of aspartate aminotransferase (AST).

Dietary instructions and bedtime uncooked cornstarch had only a partial effect on the hypoglycemia episodes, which however improved with age.

Other problems included persistent leg pain (easy fatigue and postexercise myalgia) with creatine phosphokinase (CPK) elevated up to 479 (ref. 60–365) U/L, but with normal other muscle and neurological investigations.

Genetic testing for GSD 0, VI, and XI was carried out by direct sequencing of *GYS2*, *PYGL*, and *PHKA2*, revealing a novel, hemizygous *PHKA2* variant, c.4C>G, p.(Arg2Gly), as in Families C and D; as well as a heterozygote variant of uncertain clinical significance in *PYGL*, c.859T>C, p.(Phe287Leu), not reported in HGMD or ClinVar. Additional CDG and mtDNA screening were negative.

At follow-up until 13.5 years, 4 years of growth hormone treatment under the idiopathic short stature indication normalized his height and pubertal development was normal.

The novel *PHKA2* missense variant c.4C>G was found in the older brother (II:1, Figure 1e). The brother had no history of hypoglycemia and was taller than his target height.

3.5.2 | Family investigations

The mother (I:2) had a history of hypoglycemia symptoms after prolonged fasting, but no documented hypoglycemia. She and the maternal aunt had a history of muscle pain.

3.6 | Family F

3.6.1 | The proband

In the Danish Family F, the proband (III:2, Figure 1f) presented with repeat mostly fasting KH attacks from 24 months' age (Table 1). Routine investigations excluded hormonal and metabolic causes of KH with a few exceptions. By two intramuscular (i.m.) glucagon tests, the boy's glucose only rose 1.0 (from 3.3 to 4.3) mmol/L and 0.9 (from 5.6 to 6.5) mmol/L, respectively. The initial hepatic ultrasound showed marginal hepatomegaly and hyperechogenicity, however, normalized at a repeated ultrasound at 5.5 years of age. Hepatic elastography was normal with a median of 3.5 (ref. <6) kPa and CAP medium value of 238 (ref. <250) dB/m. A 50-gene hypoglycemia NGS panel including all known GSD genes identified a hemizygous, novel *PHKA2* variant, c.403G>C, p.(Val135Leu), considered likely pathogenic (Table 2).

3.6.2 | Family investigations

The novel *PHKA2* missense variant c.403G>C was identified in the family in an X-linked pattern in four other family members (Figure 1e). Only one of the family members (I:2) had a documented history of KH symptoms in childhood. The maternal grandmother I:5 had developed obesity, T2D, elevated gamma-glutamyltransferase, occasional marginally elevated ALT (as her brother I:2) and lactate dehydrogenase (Supplemental Table 2); and liver hyperechogenicity by ultrasound.

3.7 | Family G

3.7.1 | The proband

The Canadian male proband (II:1, Figure 1g) presented at age 3.5 years with symptomatic morning time hypoglycemia (POCT glucose 2.2 mmol/L) responsive to oral carbohydrates. An 18-h formal fast, lactate, urate, transaminases, lipid profile, and liver size and echotexture by ultrasound were normal. Endocrinological evaluations were noncontributory apart from subclinical hypothyroidism. Growth parameters were normal.

He subsequently experienced multiple recurrent episodes of nocturnal hypoglycemia with night-terror-like agitated awakenings accompanied by subnormal glucometer measurements in the range of 2.0–2.9 mmol/L. He was treated with regular feeds every 2–3.5 h throughout the day and cornstarch (0.6 g/kg) in milk before bed with good results. His developmental milestone acquisition was mildly delayed; microarray was normal.

NGS hypoglycemia panel showed a novel, hemizygous variant in *PHKA2*, c.2578C>T, p.(Arg860Trp), considered likely pathogenic (Table 2). PhK activity in erythrocytes was 0.15 μmol/min/gHgb (mean normal: 1.0 μmol/min/gHgb), that is, 50% higher than the diagnostic threshold of <0.1 μmol/min/gHgb for GSD IXa.

3.8 | Family H

3.8.1 | The proband

The Danish proband (III:3, Figure 1f) was investigated 6.5 years old for intermittent leg pain. History led to suspicion of repeat KH attacks, not previously noted. On a 15-h fasting test, glucose felt to 2.3 mmol/L, BOHB 2.6 mmol/L, and plasma venous lactate 1.7 (ref. 0.5–2.1) mmol/L. I.m. glucagon test performed after fasting only led to a 1.1 mmol/L increase in glucose after 30 min. Hepatic ultrasound, muscle biopsy, and routine metabolic and hormonal screenings were normal, including growth hormone stimulations tests and synachten™ test.

A 29-gene GSD NGS panel identified a rare, hemizygous *PHKA2* DNA variant, c.1576G>A, p.(Asp526Asn), classified as of unknown clinical significance (Table 2). On follow-up until 9 years of age, the KH attacks had become milder; no longer a need for cornstarch.

3.8.2 | Family investigations

The proband's cousin (III:2) had similar KH symptoms as the proband, recognized from 20 months age, but was found wild type for the *PHKA2* variant p.(Asp526Asn). Chromosomal microarray and a 50 genes hypoglycemia panel were normal.

The proband's mother (II:5) and her elder sister (II:2) both had frequent episodes with severe nausea, uneasiness, sweating, shakiness, irritability, and relief after food, recognized since puberty (Table 3). Both of them were found to carry *PHKA2* variant p.(Asp526Asn). They both developed severe obesity and polycystic ovary syndrome in adulthood. Slimming led to an aggravation of KH symptoms. Liver biochemical parameters were occasional mildly elevated in both (Supplemental Table 2).

4 | DISCUSSION

4.1 | Major findings

In 12 KH children from 8 families, we identified two known and three (likely) pathogenic *PHKA2* DNA variants. DNA analyses on the probands and their families led to reclassification ($n = 11$), or a suggestion of reclassification ($n = 1$), of the KH diagnosis to GSD IXa. Erythrocyte PhK activity in three patients with a novel DNA variation was in the range of 15%–20% of mean normal, in contrast to the usual diagnostic threshold of <10% for GSD IXa, in keeping with a milder phenotype of KH-only GSD IXa.

4.2 | The phenotypes of IKH and GSD IXa

IKH is a frequent diagnosis found by exclusion in almost one-third (31%) of the pediatric patients older than 6 months presenting with recurrent hypoglycemia (Daly et al., 2003).

A few attempts have been made to study the pathophysiology of IKH. The glucose utilization rate did not decrease during ketogenic diet-induced hypoglycemia, in contrast to elder children without actual hypoglycemia (Dahlquist et al., 1979). The endogenous glucose production in twelve 2.5- to 11.5-year-old children with IKH during fast was significantly lower than that of elder IKH children without fasting hypoglycemia (Huidekoper et al., 2008). No differences in glycogenolysis or gluconeogenesis were found between the groups, and no children had *GYS2* variants, excluding GSD 0. In both studies, IKH was considered to be a nonpathological condition restricted to infants, representing the lower tail of the Gaussian distribution for fasting tolerance as earlier suggested (Senior, 1973).

The phenotype of GSD IXa is variable but strictly hepatic, with recurrent ketotic normoglycemia, KH, hepatomegaly, variable liver disease, retarded growth and motor development, and elevated lipids (Bali et al., 2017; Beauchamp et al., 2007; Burwinkel et al., 1998; Chen & Weinstein, 2016; Roscher et al., 2014; Tsilianidis et al., 2013; Weinstein et al., 2018). Hepatomegaly typically presents between 6 months and 2 years (Bali et al., 2017; Bhattacharya, 2015). The clinical course is usually self-limiting and most patients become asymptomatic in adulthood, but rare long-term complications include liver fibrosis, cirrhosis, adenomas, and even hepatocellular carcinoma (Bali et al., 2017; Chen & Weinstein, 2016; Roscher et al., 2014; Tsilianidis et al., 2013; Willems et al., 1990).

4.3 | Genetic-based reclassification of KH to GSD IXa and expansion of the GSD IXa phenotype

The diagnosis of GSDs is today primarily based on DNA studies rather than liver biopsy and/or erythrocyte enzyme analyses (Bali et al., 2017; Chen & Weinstein, 2016; Kishnani et al., 2019; Weinstein et al., 2018). In novel variants, additional evidence is, however, of interest. We were able to reclassify 11 IKH children from the eight families A–H to a diagnosis of phosphorylase B kinase deficiency with a mild, KH-only variant of GSD IXa, by identification of 2 known *PHKA2* DNA variants in GSD IXa, p.(Pro869Arg) and p.(Pro498Leu) (Beauchamp et al., 2007), and three novel variants p.(Arg2Gly), p.(Val135Leu), and p.(Arg860Trp).

Our three families C–E carried the same, novel *PHKA2* variant p.(Arg2Gly), which we classified as pathogenic. The prevalence of p.(Arg2Gly) in gnomAD (4.4/100,000) is high compared to the overall estimated prevalence of all GSD IX of 1/100,000 (Maichele et al., 1996). In five of our six investigated children, the p.(Arg2Gly) variant associated with documented KH in childhood. Moreover, KH symptoms in adulthood were reported in all five family members with the p.(Arg2Gly) variant. This suggests a widespread underdiagnosis of GSD IXa with a milder KH-only phenotype in both North America and Europe, if not global, owing to this one *PHKA2* variant alone.

We furthermore classified the variants p.(Val135Leu) and p.(Arg860Trp) as likely pathogenic. The patients with these variants further support a KH-only phenotype of GSD IXa, as they had ultrasound-proven normal liver size, normal liver biochemical parameters, and normal growth at last follow-up.

Finally, the novel *PHKA2* variant, p.(Asp526Asn), in the proband of Family H, was classified as a variant of uncertain significance. Despite a very similar phenotype, his cousin did not carry the *PHKA2* variant, emphasizing the complex genetics of KH with the need for broader NGS-panel testing or family exome sequencing in such patients.

None of the novel *PHKA2* DNA variants were within or close to the suggested mutational hot spot codons associated with X-linked glycogenosis 2 (XLG2, or GSD IXa2), a subtype with normal or high PhK enzyme activity in erythrocytes despite low liver PhK activity (Burwinkel et al., 1996; Burwinkel et al., 1998; Hendrickx et al., 1996). The erythrocyte enzyme activity of p.(Arg2Gly) and p.(Arg860Trp) were below normal, but higher than the GSD IXa diagnostic threshold, keeping with a mild version of the XLG1 (GSD IXa1) subtype.

An exception from the KH-only phenotype in GSD IXa was a low response to formal glucagon testing in the probands of Family E and F. A low glucose response to glucagon, which acts through activating liver PhK, is indicative of GSD IXa. The normal glucagon response in the remaining probands may be attributed to decreased, but not absent function of PhK, and variations in fasting time or glycogen stores before the glucagon test.

Another exception was the short stature and occasional mildly elevated AST in the proband of Family E. This demonstrates that the phenotype of *PHKA2* variants cannot be split into two distinct phenotypes of KH-only and classical GSD IXa with liver affection, but represents a continuum between the two.

In all our seven families with a known or novel (likely) pathogenic DNA variant, two siblings (A, III:1 and E, II:1) had a history without KH symptoms; one tested with normal fasting glucose and HbA1c age 13 years. Of the 18 family members (Table 3) with a known or novel *PHKA2* variant, at least 10 had KH-related symptoms in childhood (repeat febrile convulsions not counted), of which 8 still had mild symptoms in adulthood, whereas 8 never had reported KH symptoms.

This further expands the continuum of the phenotype of *PHKA2* variants from a seemingly symptom-free state even in childhood through KH-only to classical GSD IXa.

As an additional consequence of our family investigations, KH should not only be regarded as a disease in preschool children, although the symptoms in adulthood were mild and easy to prevent and treat.

To the best of our knowledge, only one other study has looked into the possibility of GSDs in children with IKH by genetic analysis. In 164 children with KH, 12% had presumed or known disease-causing variants in *PHKA2* (GSD IXa, $n = 12$), *GYS2* (GSD 0, $n = 4$), *PYGL* (GSD VI, $n = 2$), *PHKB* (GSD IXb, $n = 1$), or *PHKG2* (GSD IXc, $n = 1$) (Brown et al., 2015). While GSD 0 represents glycogen synthase deficiency and hence no hepatomegaly, the children with GSD VI or IX were presumed to have a missed diagnosis of hepatomegaly by ultrasound, and of abnormal liver counts, as these investigations were not performed.

Our study demonstrates the possibility of a KH-only variant of GSD IXa. In recent two Asian case reports, a novel *PHKA2* variant of uncertain significance, c.2972C>G, p.(Gly991Ala), has been found in a Chinese and a Japanese child with KH as the only manifestation (Ago et al., 2019; Fu et al., 2019). The Japanese child had normal phosphorylase activity, and the prevalence of the variant in Japan was found to be 1:200 in males but elsewhere rare (Ago et al., 2019). More data are needed on this *PHKA2* variant to establish it as the cause of a suggested high frequency of KH-only GSD IXa in East Asia.

As in other X-linked diseases, the GSD IXa phenotype is usually more pronounced in males. In our series, 9 boys and 3 girls had KH-GSD IXa, but no sex difference in disease severity was detectable. An investigation for skewed X chromosome inactivation in one of our most affected adult females (C, II:7) was negative, this does however not exclude somatic skewed inactivation in the liver.

4.4 | Other aspects

In the male proband of Family B (IV:1), follow-up at 18 years' age showed hyperechogenicity and moderately increased CAP value by elastography. It is not clear, whether the increased CAP value represented steatosis, increased glycogen stores or both. Liver biopsies in other GSD IX patients have shown both increased glycogen and steatosis in hepatocytes (Bali et al., 2017; Tsilianidis et al., 2013). To the best of our knowledge, the use of elastography has not been reported in patients with GSD IX but it may be a valuable tool in detecting early hepatic changes in GSD IX.

Moreover, two adults with a known or likely pathogenic *PHKA2* variant (B, IV:5 and F, I:5) had hyperechogenicity and at least occasionally elevated ALT. The liver infection in these three individuals indicates a need for prolonged follow-up and family investigations in KH-only GSD IXa to identify late-presenting liver affection, which may demand dietary management despite the absence of KH in adulthood.

In Family F, I:2 and I:5 had T2D at the age of 76 and 70 years, respectively. This may raise a concern of T2D as a consequence of recurrent hypoglycemia, leading to overeating and obesity in KH and undiscovered GSD IXa. Prolonged follow-up and family investigations are encouraged in patients with GSD IXa as stated by others (Bhattacharya, 2015; Wolfsdorf & Weinstein, 2003).

The youngest brother in Family B (IV:2) had severe neurosensory hearing loss diagnosed at 10 months age. Hearing loss in GSD IXa has been described once before in a patient with additional neurological manifestations and was interpreted as a coincident finding (Burwinkel et al., 1998). On the other hand, neurosensory hearing loss is frequently seen in classical GSD II, presumably caused by progressive storage of glycogen in the inner ear (van Capelle et al., 2010). Intensive genetic investigations including WES failed to identify a cause of the congenital hearing loss in our patient.

In both affected brothers in Family B (IV:1 and IV:2), elevated plasma pyruvate was seen together with elevated lactate and triglycerides upon attacks of KH. Measurement of plasma pyruvate has to the best of our knowledge never been reported in either IKH or GSD IX. Increased pyruvate concentrations may be a clue for GSD IXa in infants with IKH.

One of our patients (Family E, II:2) had a report of persistent leg pain, easy fatigue and post-exercise myalgia with mildly elevated CPK. This may be attributed to his additional heterozygous *PYGL* DNA variant, although GSD VI is only known to be caused by biallelic DNA variants in *PYGL*. Although unexplained KH may hypothetically be caused by DNA variants in two different genes involved in GSD, no data points toward a digenic cause of KH in this patient.

Apart from Patient E, II:2, none of our children presented growth retardation, muscular weakness, or other aspects of GSD IXa. This may reflect a milder phenotype or an earlier diagnosis and treatment of KH as stated by others (Chen & Weinstein, 2016; Daly et al., 2003; Tsilianidis et al., 2013).

However, the hypoglycemia reached 1.1 mmol/L in our series, and the diagnosis was not always early. Nevertheless, our findings support the urge for adequate diagnosis and treatment of KH patients in opposition to considering IKH as the lower tail of normal variance.

4.5 | Strengths and limitations

Our study had the strength of including multicenter data, detailed phenotyping with a long follow-up time, and family member data. Limitations included the retrospective data capture with lack of, for example, precise fasting time prior to glucagon tests, missing liver biopsy data, and the lack of age-matched normative data for all biochemical measurements.

5 | CONCLUSION

Our study expands the phenotype of GSD IXa to a continuum from a seemingly asymptomatic state, over KH-only, to more or less complete classical GSD IXa. Patients with a diagnosis or suspicion of IKH should be investigated for *PHKA2* variants, suitably performed in NGS panels for hypoglycemia or GSD, to improve the precision of treatment and prognosis, and to diagnose affected family members.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data from this study are available on request.

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