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RESEARCH ARTICLE

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Expanding the genotypic and phenotypic spectrum of severe serine biosynthesis disorders

Fatima Abdelfattah[1] | Ariana Kariminejad[2] | Anne-Karin Kahlert[3,4] | Patrick J. Morrison[5] | Evren Gumus[6] | Katherine D. Mathews[7] | Benjamin W. Darbro[7] | David J. Amor[8,9,10] | Maie Walsh[8] | Yves Sznajer[11] | Luisa Weiß[3] | Sabine Weidensee[12] | David Chitayat[13,14] | Patrick Shannon[15] | Eva Bermejo-Sánchez[16] | Isolina Riaño-Galán[17,18,19] | Ian Hayes[20] | Gemma Poke[21] | Caroline Rooryck[22] | Perrine Pennamen[22] | Suonavy Khung-Savatovsky[23] | Annick Toutain[24] | Marie-Laure Vuillaume[24] | Siavash Ghaderi-Sohi[2] | Mohamad H. Kariminejad[2] | Sönke Weinert[25] | Heinrich Sticht[26] | Martin Zenker[1] | Denny Schanze[1]

[1]Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Germany

[2]Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran

[3]Institut für Klinische Genetik, Medizinische Fakultät Carl Gustav Carus, Technische Universität Dresden, Dresden, Germany

[4]Department of Congenital Heart Disease and Pediatric Cardiology, University Hospital Schleswig-Holstein, Kiel, Germany

[5]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, UK

[6]Division of Medical Genetics, School of Medicine, Harran University, Sanliurfa, Turkey

[7]Carver College of Medicine, University of Iowa, Iowa City, Iowa

[8]Murdoch Children's Research Institute, Royal Children's Hospital, Parkville, Victoria, Australia

[9]Department of Paediatrics, The University of Melbourne, Melbourne, Victoria, Australia

[10]Royal Children's Hospital, Parkville, Victoria, Australia

[11]Centre de Génétique Humaine, Cliniques Universitaires Saint Luc, Université Catholique de Louvain, Brussels, Belgium

[12]Human Medical Genetics, Erfurt, Germany

[13]Department of Obstetrics and Gynecology, The Prenatal Diagnosis and Medical Genetics Program, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada

[14]Division of Clinical and Metabolic Genetics, The Hospital for SickKids, University of Toronto, Toronto, Ontario, Canada

[15]Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada

[16]ECEMC (Spanish Collaborative Study of Congenital Malformations), Research Unit on Congenital Anomalies (UIAC), Institute of Rare Diseases Research (IIER), Institute of Health Carlos III, Ministry of Science and Innovation, Madrid, Spain

[17]AGC de Pediatría, Hospital Universitario Central de Asturias, Oviedo, Spain

[18]IUOPA-Departamento de Medicina-ISPA, Universidad de Oviedo, Oviedo, Spain

[19]CIBER de Epidemiologia y Salud Pública, Madrid, Spain

[20]Genetic Health Service New Zealand, Auckland Hospital, Auckland, New Zealand

[21]Genetic Health Service New Zealand, Wellington Regional Hospital, Wellington, New Zealand

[22]MRGM INSERM U1211, CHU de Bordeaux, Service de Génétique Médicale, University of Bordeaux, Bordeaux, France

[23]Unité de Fœtopathologie, Hôpital Universitaire Robert-Debré, Paris, France

[24]Service de Génétique, CHU de Tours, UMR 1253, iBrain, Université de Tours, INSERM, Tours, France

[25]Department of Cardiology and Angiology, Internal Medicine, University Hospital Magdeburg, Magdeburg, Germany

[26]Institute of Biochemistry, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

Correspondence
Martin Zenker, Institute of Human Genetics, University Hospital Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany.
Email: martin.zenker@med.ovgu.de

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Abstract

Serine biosynthesis disorders comprise a spectrum of very rare autosomal recessive inborn errors of metabolism with wide phenotypic variability. Neu–Laxova syndrome represents the most severe expression and is characterized by multiple congenital anomalies and pre- or perinatal lethality. Here, we present the mutation spectrum and a detailed phenotypic analysis in 15 unrelated families with severe types of serine biosynthesis disorders. We identified likely disease-causing variants in the *PHGDH* and *PSAT1* genes, several of which have not been reported previously. Phenotype analysis and a comprehensive review of the literature corroborates the evidence that serine biosynthesis disorders represent a continuum with varying degrees of phenotypic expression and suggest that even gradual differences at the severe end of the spectrum may be correlated with particular genotypes. We postulate that the individual residual enzyme activity of mutant proteins is the major determinant of the phenotypic variability, but further functional studies are needed to explore effects at the enzyme protein level.

KEYWORDS
autosomal recessive, genotype–phenotype correlation, L-serine biosynthesis, Neu–Laxova syndrome, PHGDH, PSAT1

1 | INTRODUCTION

Serine biosynthesis disorders constitute a clinically and genetically heterogeneous group of autosomal recessive diseases caused by pathogenic changes in genes encoding enzymes of the L-serine biosynthesis pathway: phosphoglycerate dehydrogenase (*PHGDH*; MIM# 606879), phosphoserine aminotransferase (*PSAT1*; MIM# 610936), and phosphoserine phosphatase (*PSPH*; MIM# 172480; Acuna-Hidalgo et al., 2014; Hart et al., 2007; Klomp et al., 2000; Shaheen et al., 2014; Veiga-da-Cunha et al., 2004). The phenotype ranges from nonspecific developmental delay (Tabatabaie et al., 2011) to the severe lethal disease known as Neu–Laxova syndrome (NLS; MIM# 256520). Following the first descriptions by Neu and Laxova in 1971 and 1972, respectively (Laxova, Ohara, & Timothy, 1972; Neu, Kajii, Gardner, & Nagyfy, 1971), fewer than 100 cases of NLS have been reported to date (Acuna-Hidalgo et al., 2014; Bourque et al., 2019; Cavole et al., 2020; Coto-Puckett et al., 2010; El-Hattab et al., 2016; Manning, Cunniff, Colby, El-Sayed, & Hoyme, 2004; Mattos et al., 2015; Ni et al., 2019; Shaheen et al., 2014). The clinical hallmarks of this disorder are severe intrauterine growth restriction (IUGR), microcephaly, cutaneous abnormalities, and distinctive craniofacial features including sloping forehead, ocular hypertelorism, prominent eyes, ectropion, flat nose, round gaping mouth, micrognathia, short neck, and low-set malformed ears. Variable central nervous system (CNS) abnormalities have been described including microcephaly, lissencephaly, hypoplastic cerebellum, agenesis, or dysgenesis of the corpus callosum, and Dandy–Walker malformation (Badakali, Badakali, & Dombale, 2012; Coto-Puckett et al., 2010; Ostrovskaya & Lazjuk, 1988). The spectrum of skin abnormalities includes varying degrees of ichthyosis (Curry, 1982), edema of the hands and feet, and excessive fatty tissue under the epidermis (Karimi-Nejad, Khajavi, Gharavi, & Karimi-Nejad, 1987). Joint contractures are common and pterygia may be present, most likely reflecting fetal akinesia (Curry, 1982; Ejeckam, Wadhwa, Williams, & Lacson, 1986; Fitch, Resch, & Rochon, 1982). Additional congenital malformations including cleft lip and palate (Coto-Puckett et al., 2010; Rouzbahani, 1995), spina bifida (Manning et al., 2004; Naveed, Manjunath, & Sreenivas, 1990), genitourinary anomalies (cryptorchidism, hypoplastic genitalia, renal agenesis; Naveed et al., 1990; Shivarajan, Suresh, Jagadeesh, Lata, & Bhat, 2003), pulmonary and gastric hypoplasia (Manning et al., 2004) have occasionally been reported. Although pre- or perinatal lethality is a hallmark of this condition, survival from a few weeks to several months has been observed in some cases (Carder, Fitzpatrick, & Weston, 2003; Coto-Puckett et al., 2010; El-Hattab et al., 2016; Horn, Muller, Thiele, & Kunze, 1997; Ugras, Kocak, & Ozcan, 2006), presumably representing milder variants of the syndrome. So far, only 12 different variants in the *PHGDH* gene, 5 in the *PSAT1* gene, and 1 frameshift variation in *PSPH* have been identified in association with NLS (Tables S1–S3 and Figure 1).

The nonlethal forms of serine biosynthesis deficiency comprise nonspecific disorders with neurodevelopmental defects, epilepsy, and

microcephaly as the major clinical findings and low serine levels in plasma and cerebrospinal fluid as the diagnostic hallmark (Brassier et al., 2016; Byers et al., 2016; de Koning et al., 1998; El-Hattab et al., 2016; Glinton et al., 2018; Hart et al., 2007; Hausler, Jaeken, Monch, & Ramaekers, 2001; Jaeken et al., 1996; Klomp et al., 2000; Meneret et al., 2012; Pind et al., 2002; Pineda et al., 2000; Poli et al., 2017; Tabatabaie et al., 2009, 2011; Veiga-da-Cunha et al., 2004; Vincent et al., 2015). A spectrum of disease-causing variants in the same genes have been reported in the nonlethal forms, which differs from NLS-associated variants (Tables S1–S3 and Figure 1). It was proposed that the severity of enzymatic deficiency is the main cause of phenotypic variability of serine biosynthesis disorders, and considerable residual enzymatic activity has been demonstrated for some variants associated with nonlethal serine deficiency.

Herein, we report a new cohort of patients with severe serine biosynthesis disorders, thereby expanding and further elucidating the genotype–phenotype spectrum.

2 | MATERIALS AND METHODS

2.1 | Patients

Patients referred for molecular genetic testing for suspected NLS/severe serine biosynthesis disorder or with a diagnosis of such a disease established by whole exome sequencing (WES) were eligible for this study. All molecular testing was performed in a diagnostic context after obtaining informed consent according to the respective national regulations for genetic testing. Specific written parental permission was obtained for publication of photographs presented in this article.

The study cohort included a total of 19 patients, 10 females and 9 males, from 15 unrelated families. Three of these families had a history of another probably affected offspring, but no details were available from these. The clinical data of all patients were collected using a questionnaire. Families were of various ethnic origin (Table 1). The majority of the patients were stillborn or died shortly after birth. In five cases, the pregnancy was terminated after severe malformations having been identified by fetal ultrasonography.

2.2 | Molecular analysis

DNA was extracted from blood samples of the patients, except for families 4 and 11, where only FFPE (formalin-fixed, paraffin-embedded) tissue from the affected fetus was available. From three families, DNA samples of affected fetuses (patients 5a/5b, 12, and 14) were not available, and therefore parental DNA was tested assuming heterozygous carrier status (Table 1). In family 5, a chorionic villus sample DNA (CVS DNA) was analyzed to provide prenatal diagnosis in a family with a previous child clinically diagnosed with NLS.

In the majority of samples, targeted Sanger sequencing was performed for all coding exons and flanking intronic regions of the known causative genes for NLS (*PHGDH* [ENST00000369409]; *PSAT1* [ENST00000376588]; *PSPH* [ENST00000395471]). Sequence data were generated using the ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Germany) and an automated capillary sequencer (ABI 3500; Applied Biosystems), and Sequence Pilot software (JSI medical systems, Germany) was used for analysis. Due to the very poor quality of the DNA extracted from the formalin-fixed tissue of patients 4 and 11, no full mutation screening of the three genes of interest was possible. Only amplification of a very short fragment of exon 7 of *PHGDH* and of exon 4 of *PSAT1*, encompassing the most common NLS-associated variant in this gene, were successful, respectively. In four cases (families 4, 6, 7, and 8) the molecular diagnosis was made by diagnostic (trio) exome sequencing. All variants identified in the three genes were analyzed using the online prediction tools MutationTaster (http://www.mutationtaster.org/), Meta-SNP (http://snps.biofold.org/meta-snp/), and CADD (https://cadd.gs.washington.edu/). Human splicing finder (http://www.umd.be/HSF3/) was used to predict the effect of the splice site variants. Mutated protein stability was also analyzed in silico using an integrated predictor for protein stability change upon single mutation (http://predictor.nchu.edu.tw/istable/). The level of evidence for pathogenicity of all observed variants was classification according to the recommendations of the American College of Medical Genetics and Genomics (ACMG; Richards et al., 2015). All the variants were submitted to the genetic Leiden Open Variation Database, LOVD v.3.0 Build 23 (https://www.lovd.nl/).

2.3 | Structural analysis and molecular modeling

The structural analysis of the protein variants was performed based on the crystal structures of PHGDH (PDB code: 2G76) and PSAT1 (PDB code: 3E77). Missense changes were modeled with SwissModel (Guex & Peitsch, 1997) and RasMol (Sayle & Milner-White, 1995) was used for structure analysis and visualization.

3 | RESULTS

3.1 | Mutation screening

In 15 families with NLS, variants in either *PSAT1* or *PHGDH* were identified that were considered as likely causative (Table 2). In one of these families (family 4) with abnormal fetuses occurring in two branches of the family, which was previously reported negative for a mutation in the three genes for NLS (family 11 in Acuna-Hidalgo et al., 2014), exome sequencing of additional family members revealed a likely disease-causing *PHGDH* variant in one branch of the family (Figure S1). In total, the disease was attributable to *PSAT1* in 11 unrelated families and to *PHGDH* in 4 (Table 1). *PSPH* variants were not observed in this cohort. A total of 13 different presumably disease-causing variants were identified in those two genes. Nine of

TABLE 1 Genotype, family information, auxology, and major malformations in the study cohort

Patient/family number	1	2	3	4	5a	5b
Mutated gene	PHGDH	PHGDH	PHGDH	PHGDH	PSAT1	PSAT1
Nucelotide change (status)	c.160C>T (hom)	c.488G>A (hom)	c.638C>T (hom; UPD1)	c.704C>T (hom)	c.1A>G (presumed hom; het in parents)	c.1A>G (presumed hom; het in parents)
Predicted effect on RNA/protein	p.(Arg54Cys)	p.(Arg163Gln)	p.(Thr213Met)	p.(Ala235Val)	p.?	p.?
Mutation detection method	TS	TS	TS	WES	TS	TS
Gender	Female	Female	Female	Male	Female	Female
Parental consanguinity	Yes			First cousins	First cousins	First cousins
Affected siblings				Possibly affected fetus in previous pregnancy	Sister	Sister
Ethnic background	Caucasian (France)	Pakistan	NA	Iranian	Iranian	Iranian
Gestational age at birth	26 + 6 weeks	39 + 2 weeks	40 weeks	33 weeks	40 weeks	40 weeks
Birth weight	379 g (−2.5 *SD*)	1,988 g (−3.2 *SD*)	2,540 g (−2.0 *SD*)	1,179 g (−2.1 *SD*)	1,900 g (−3.7 *SD*)	2,200 g (−2.9 *SD*)
Body length at birth	28 cm (−2.7 *SD*)	42.5 cm (−3.1 *SD*)	39 cm (−5.0 *SD*)	31 cm (−4.9 *SD*)	40 cm (−4.6 *SD*)	39 cm (−5.0 *SD*)
OFC at birth	18 cm (−4.5 *SD*)	27.4 cm (−5.0 *SD*)	28.2 cm (−4.7 *SD*)	26 cm (−2.9 *SD*)	30 cm (−3.4 *SD*)	29 cm (−4.1 *SD*)
Survival/death	TOP	Liveborn, died on 1st day	Liveborn, died after 30 days	Stillborn	Liveborn, died after 27 days	Liveborn, died after 5 days
Cleft lip/cleft palate						
Central nervous system abnormalities	LIS, CH, CAL, HYD	LIS, CH, CAL, HYD	LIS, CH, CAL, HYD	LIS, CAL	NA	NA
Limb and skeletal anomalies	CON	CON, EDE	CON, EDE	CON, EDE	CON	CON
Skin abnormalities	ICH	RDERM	Mild ICH	RDERM	ICH	ICH
Genitourinary abnormalities	GEN		KID	GEN		
Additional anomalies	Cataract	Cataract			Cataract	Cataract

Patient/family number	6a	6b	7a	7b	8	9
Mutated gene	PSAT1	PSAT1	PSAT1	PSAT1	PSAT1	PSAT1
Nucelotide change (status)	c.129T>G (hom)	c.129T>G (presumed hom; no gene analysis in this patient)	c.181C>T (het, mat) c.296C>T (het, pat)	c.181C>T (het, mat) c.296C>T (het, pat)	c.235G>T (hom)	c.296C>T (hom)
Predicted effect on RNA/protein	p.(Ser43Arg)	p.(Ser43Arg)	p.(Arg61Trp) p.(Ala99Val)	p.(Arg61Trp) p.(Ala99Val)	p.(Gly79Trp)	p. (Ala99Val)

TABLE 1 (Continued)

Patient/family number	6a	6b	7a	7b	8	9
Mutation detection method	WES	No gene analysis	WES	TS	WES	TS
Gender	Male	Male	Female	Male	Female	Male
Parental consanguinity	First cousins	First cousins			2nd degree cousins	3rd degree cousins
Affected siblings	Brother	Brother	Male sibling fetus	Sister	Probably affected male sibling	
Ethnic background	Turkish	Turkish	Caucasian (German)	Caucasian (German)	Arab	Arab (Algeria)
Gestational age at birth	36 weeks	35 weeks	32 + 2 weeks	15 weeks	26 weeks	36 + 5 weeks
Birth weight	2,500 g (−1.7 *SD*)	NA	820 g (−2.4 *SD*)	33 g	278 g (−3.2 *SD*)	1,330 g (−3.8 *SD*)
Body length at birth	47 cm (−1.1 *SD*)	NA	33 cm (−3.3 *SD*)	9 cm	NA	33 cm (−6.2 *SD*)
OFC at birth	30 cm (−2.3 *SD*)	NA	24 cm (−3.4 *SD*)	NA	NA	25 cm (−5.5 *SD*)
Survival/death	Alive (age 4 years)	Died at age 6.5 years	Liveborn, died after 4 months	TOP	TOP	Liveborn, died on 1st day
Cleft lip/cleft palate	CP	CP	CP			
Central nervous system abnormalities	CAL, HYD	NA	CAL, HYD	NA	LIS, CH, CAL, HYD	LIS, CAL, HYD
Limb and skeletal anomalies	CON, EDE	CON	CON	EDE	CON, EDE	CON, EDE
Skin abnormalities	Mild ICH	NA		NA		ICH
Genitourinary abnormalities	GEN	NA	GEN			GEN, KID
Additional anomalies	Cataract	Cataract	Duodenal atresia, polydactyly	Pectus excavatum, gut malformation		VSD, sacral agenesis

Patient/family number	10	11	12	13	14	15a	15b
Mutated gene	PSAT1	PSAT1	PSAT1	PSAT1	PSAT1	PSAT1	PSAT1
Nucelotide change (status)	c.296C>T (het) c.870-1G>T (het)	c.296C>T (het, mat) Undetected 2nd allele	c.733T>C (presumed hom; het in parents)	c.463G>C (het) c.870-1G>T (het)	c.870-1G>T (presumed hom; het in parents)	c.955delA (hom)	c.955delA (hom)
Predicted effect on RNA/protein	p.(Ala99Val) Splicing	p.(Ala99Val)	p.(Cys245Arg)	p.(Glu155Gln) Splicing	Splicing	p.(Arg319Aspfs*14)	p.(Arg319Aspfs*14)
Mutation detection method	TS	TS	TS	TS	TS	TS	TS

(Continues)

TABLE 1 (Continued)

Patient/family number	10	11	12	13	14	15a	15b
Gender	Male	Female	Male	Female	Female	Male	Male
Parental consanguinity		NA	2nd degree cousins				
Affected siblings			Probably affected male sibling			Brother	Brother
Ethnic background	NA	NA	Turkish	NA	Caucasian (Spain)	Tuvalu	Tuvalu
Gestational age at birth	37 + 2 weeks	39 weeks	32 weeks	38 + 5 weeks	35 weeks	31 + 6 weeks[a]	18 + 6 weeks
Birth weight	1,371 g (−4.0 *SD*)	2,120 g (−2.9 *SD*)	770 g (−2.6 *SD*)	1,507 g (−4.6 *SD*)	NA	1,690 g (−0.2 *SD*)	96.2 g
Body length at birth	38.6 cm (−4.8 *SD*)	NA	30 cm (−4.7 *SD*)	NA	NA	43 cm (+0.5 *SD*)	17.8 cm
OFC at birth	24.6 cm (−6.1 *SD*)	NA	20.5 cm (−6.0 *SD*)	26.4 cm (−5.4 *SD*)	NA	29 cm (−0.2 *SD*)	11.6 cm
Survival/death	Liveborn, died after 5 days	Liveborn, died after 7 days	TOP	Liveborn, died on 1st day	Stillborn	Liveborn, died on 1st day	TOP
Cleft lip/cleft palate				CP			CP
Central nervous system abnormalities	LIS, CH, CAL, HYD	NA	LIS, CH, CAL	LIS, CH, HYD	NA	CH	CH
Limb and skeletal anomalies	CON	CON, EDE	CON	CON, EDE	CON, EDE	CON, EDE	CON
Skin abnormalities	RDERM	RDERM	ICH	RDERM	RDERM	RDERM	ICH
Genitourinary abnormalities	GEN		KID		GEN	GEN, KID	KID
Additional anomalies	Cataract		Cataract, AVSD, situs inversus	Myocardial hypertrophy			

Note: A more detailed compilation of clinical data is provided in Table S4.

Abbreviations: AVSD, atrioventricular septal defect; CAL, callosal hypoplasia or agenesis; CH, cerebellar hypoplasia; CLP, cleft lip and cleft palate; CON, joint contractures; CP, cleft palate; EDE, edema/swelling of hands and/or feet; GEN, genital hypoplasia/anomalies; het, heterozygous; hom, homozygous; HYD, hydrocephalus/enlarged ventricles; ICH, ichthyosis; KID, kidney anomalies, LIS, lissencephaly spectrum; mat, maternal; OFC, occipital frontal circumference; pat, paternal; RDERM, restrictive dermopathy; SD, standard deviation; TOP, termination of pregnancy; TS, targeted sequencing; VSD, ventricular septal defect; WES, whole exome sequencing.

[a]Estimation of gestational age in P15a was based on fetal size at the first scan, which correlated with a gestational age of 21 + 4 weeks; it is therefore probably underestimated.

them were novel (previously not reported in NLS). Two *PSAT1* variants were recurrent in this cohort: one of the previously reported variants (c.296C>T, p.(Ala99Val)); observed in four unrelated families) and one novel splice acceptor change (c.870-1G>T; observed in three unrelated families). The novel *PHGDH* and *PSAT1* variants were either absent from the gnomAD database (https://gnomad.broadinstitute.org/) or present at a very low frequency, compatible with the expected carrier frequency for a very rare recessive disease. The highest allele frequency of 1.52e−4 was recorded for the recurrent *PSAT1* variant (c.296C>T, p.(Ala99Val)). Three of the novel missense variants received a formal classification of VUS (variant of uncertain significance) according to ACMG recommendations (Richards et al., 2015), while all other observed variants were classified as either pathogenic or likely pathogenic. The results of the analysis of all the novel variants by various in silico prediction tools are summarized in Table 2.

Affected individuals from six families with known parental consanguinity were homozygous for the variant considered as causative, while three families had different compound heterozygous constellations (families 7, 10, and 13). In the non-consanguineous family 3, SNP (single nucleotide polymorphism) microarray showed loss of heterozygosity for the entire length of chromosome 1, indicating that homozygosity for the *PHGDH* variant resulted from UPD of chromosome 1. In one family (family 11), where paternal DNA was unavailable and the fetal material derived from FFPE tissue insufficient for a full screening, only the maternally inherited *PSAT1* variant could be identified, allowing the attribution of this case to PSAT1 deficiency (Table 1).

The distribution of novel and previously described disease-associated variants is shown in Figure 1. Notably, most NLS-associated *PHGDH* variations predicting missense changes affect the nucleotide binding domain (NBD) and the substrate binding domain (SBD) of the protein respectively, whereas the majority of the *PHGDH* variants previously observed in nonlethal PHGDH deficiency are located in the C-terminal regulatory domain (Figure 1a). In contrast, for pathogenic *PSAT1* variants no obvious phenotype-specific distribution was observed (Figure 1b).

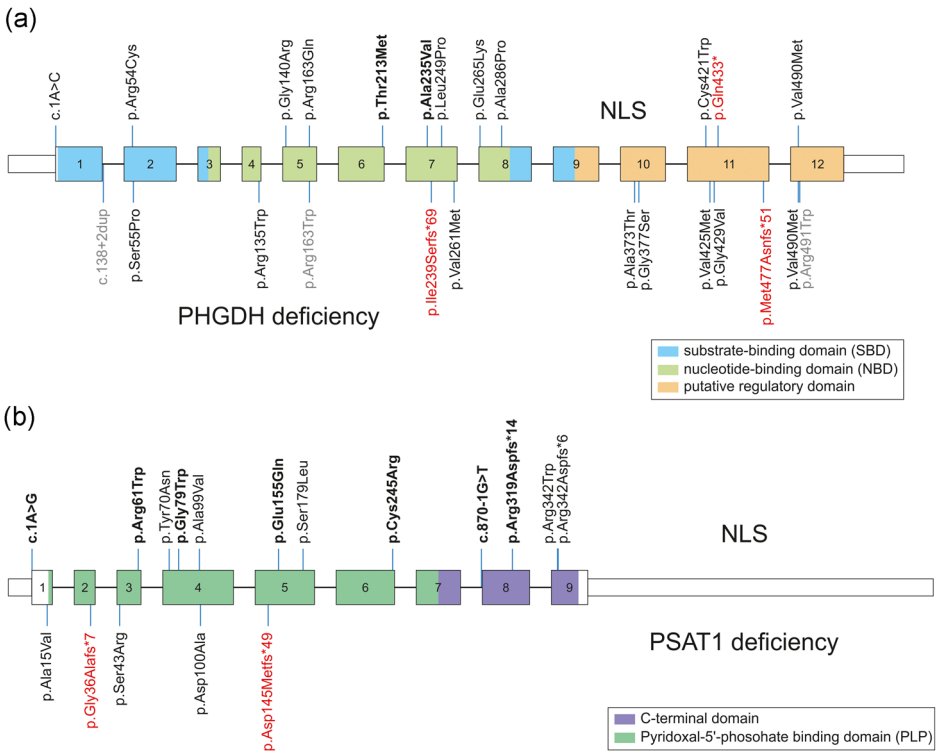

FIGURE 1 Distribution of mutations along *PHGDH* and *PSAT1* genes. Schematic representation of variants in the genes *PHGDH* (a) and *PSAT1* (b). Exons are to scale (larger rectangles represents coding region, lower represents UTR) joined by a continuous line (introns, not to scale). Different colors refer to the different domains of the protein as indicated. Disease-associated variants are shown with their locations along the genes. Variants observed in NLS are depicted above the diagrams, while variants observed in the nonlethal forms of PHGDH and PSAT1 deficiency, respectively, are shown below the diagrams. Novel variants are depicted in bold. Alterations predicting a loss-of-function (putative null alleles) are printed in red color. Variants whose functional impact could not be classified are printed in grey color (see Figures S1–S3). [Prediction of different domains of PHGDH are based on the knowledge of the 94% similarity between rat and human 3-phosphoglycerate dehydrogenase (3-PGDH) and the paralogous enzyme of *Escherichia coli*, sharing 30% identical amino acids with human 3-PGDH (Achouri et al., 1997; Cho, Jun, Bae, Ahn, & Kim, 2000; Klomp et al., 2000; Pind et al., 2002; Tabatabaie et al., 2009). Prediction of different domains of PSAT1 are based on the knowledge of the extensive homology between *E. coli* SerC and phosphoserine aminotransferase of rabbit and human that share 93.5% of their amino acid sequence (Hester et al., 1999; van der Zel, Lam, & Winkler, 1989)]. NLS, Neu–Laxova syndrome; PHGDH, phosphoglycerate dehydrogenase; PSAT1, phosphoserine aminotransferase; UTR, untranslated region

TABLE 2 PHGDH and PSAT1 variants observed in the present cohort

Gene	Variant	Consequence on RNA/protein	gnomAD[a]	Meta-SNP	MutationTaster	CADD[b]	iStable	3D modeling	ACMG[c]	LOVD³ DB-ID
PHGDH	c.160C>T	p.(Arg54Cys)	5/0	Disease causing (0.889)	Disease causing (0.999)	25.3	Decrease (0.6923)	Near substrate binding site, may cause reduced substrate affinity	LPATH (PM2, PM3, PP2, PP3)	PHGDH_000021
PHGDH	c.488G>A	p.(Arg163Gln)	Not found	Disease causing (0.683)	Disease causing (0.999)	33.0	Decrease (0.8418)	Predicted to affect dimer stability	LPATH (PS4, PM2, PP2, PP3)	PHGDH_000002
PHGDH	**c.638C>T**	**p.(Thr213Met)**	2/0	Disease causing (0.767)	Disease causing (0.999)	28.8	Decrease (0.7492)	Predicted to hamper NAD^+ binding	VUS (PM2, PP2, PP3)	PHGDH_000018
PHGDH	**c.704C>T**	**p.(Ala235Val)**	Not found	Disease causing (0.826)	Disease causing (0.999)	27.4	Decrease (0.8443)	Predicted to hamper NAD^+ binding	VUS (PM2, PP2, PP3)	PHGDH_000020
PSAT1	**c.1A>G**	**p.?**	Not found	NA	NA	24.8	NA	NA	LPATH (PVS1, PM2)	PSAT1_000009
PSAT1	c.129T>G	p.(Ser43Arg)	Not found	Disease causing (0.897)	Disease causing (0.999)	23.0	Decrease (0.7772)	Predicted to affect dimer stability	LPATH (PS4, PM2, PP2, PP3)	PSAT1_000017
PSAT1	**c.181C>T**	**p.(Arg61Trp)**	24/0	Disease causing (0.846)	Disease causing (0.999)	29.9	Decrease (0.8558)	Predicted to destabilize the enzyme fold by steric clash	LPATH (PS3, PM2, PP1, PP2, PP3)	PSAT1_000010
PSAT1	**c.235G>T**	**p.(Gly79Trp)**	Not found	Disease causing (0.862)	Disease causing (0.999)	31.0	Increase (0.6263)	Predicted to cause steric clashes with PLP	VUS (PM2, PP2, PP3)	PSAT1_000011
PSAT1	c.296C>T	p.(Ala99Val)	43/0	Disease causing (0.547)	Disease causing (0.999)	24.2	Decrease (0.7616)	Predicted to lead to protein instability	PATH (PS3, PS4, PM2, PM3, PP1, PP2, PP3)	PSAT1_000016
PSAT1	**c.463G>C**	**p.(Glu155Gln)**	1/0	Disease causing (0.846)	Disease causing (0.999)	28.4	Decrease (0.8194)	Predicted to affect PLP binding	LPATH (PM2, PM3, PP2, PP3)	PSAT1_000014
PSAT1	**c.733T>C**	**p.(Cys245Arg)**	8/0	Disease causing (0.825)	Disease causing (0.999)	33.0	Increase (0.5808)	Predicted to cause steric clashes with V39 and M42, may reduce dimer stability	LPATH (PS3, PM2, PP2, PP3)	PSAT1_000013
PSAT1	**c.870-1G>T**	**p.?**[d]	5/0	NA	NA	26.5	NA	NA	PATH (PVS1, PM2, PM3)	PSAT1_000012

TABLE 2 (Continued)

Gene	Variant	Consequence on RNA/protein	gnomAD[a]	Meta-SNP	MutationTaster	CADD[b]	iStable	3D modeling	ACMG[c]	LOVD[3] DB-ID
PSAT1	**c.955del**	**p.(Arg319Aspfs*14)**	Not found	NA	Disease causing (1.0)	35.0	Decrease (0.7891)	NA	PATH (PVS1, PM2, PP1)	PSAT1_000015

Note: Variants refer to the reference sequences of PHGDH (NM_006623.4; NG_009188.1) and PSAT1 (NM_058179.4; NG_012165.1). Novel mutations are printed in bold.

Abbreviations: ACMG, American College of Medical Genetics and Genomics; CADD, Combined Annotation Dependent Depletion; LOVD, Leiden Open Variation Database; NA, not applicable; LPATH, likely pathogenic; PATH, pathogenic; PHGDH, phosphoglycerate dehydrogenase; PLP, pyridoxal phosphate; PSAT1, phosphoserine aminotransferase; VUS, variant of uncertain significance.

[a]Representation in gnomAD is given as number of observed alleles/number of homozyotes.

[b]CADD PHRED score: CADD (http://cadd.gs.washington.edu/) v1.4 PHRED-like (−10 × log10(rank/total)) scaled C-score: ranking a variant relative to all possible substitutions of the human genome (8.6×10^9). A scaled C-score ≥ 10: variant belongs to 10% most deleterious variants; C-score ≥ 20: variant belongs to 1% most deleterious variants.

[c]Classification of pathogenicity according to Richards et al. (2015).

[d]Variant is assumed to affect splicing.

3.2 | Structural analysis and molecular modeling

To better understand the effects of the novel variants on the protein structure and functional consequences, we conducted structure analysis and molecular modeling of the novel NLS-associated missense changes in comparison to known PHGDH and PSAT1 deficiency-associated mutants. For both, PHGDH and PSAT1, the functionally active enzyme proteins are dimers, and their dimeric configuration is known to be essential for their appropriate function (John, 1995; Mishra, Ali, Nozaki, & Bhakuni, 2010).

Taken together, the structural analyses suggest that the majority of the previously reported and newly detected PHGDH and PSAT1 missense variants cluster at distinct sites of the protein structure. The major region that is affected by disease-associated variants is the substrate/cofactor binding site of the respective proteins, suggesting that the respective mutants directly affect enzymatic activity. A second hot spot region in both proteins is the subunit interface in the homodimeric enzymes, indicating that the respective variants indirectly affect activity via a reduced dimer stability. However, it must be noted that there also exist some additional variants at other enzyme sites, which may affect enzymatic activity by different mechanisms, like, for example, a reduced overall stability of the domain structure (Table 2 and Supporting Information Results and Figures S2 and S3).

3.3 | Phenotype analysis

The physical findings in 19 affected individuals with *PHGDH* and *PSAT1* variations are summarized in Table 1, and are provided in more detail in Table S4. Phenotypic features of selected patients are shown in Figure 2. All cases except the two affected children from family 6 represented pre- or perinatal deaths with an unambiguous clinical diagnosis of NLS. Four individuals died shortly after birth, two were stillborn, and in five cases, the pregnancy was terminated upon the detection of severe fetal anomalies by ultrasound or after genetic confirmation of recurrence of the disease in the fetus (patients 1, 7b, 8, 12, and 15b). The remaining individuals diagnosed as typical NLS survived up to 4 months. The median age at death of the liveborn patients with NLS (excluding family 6) was 5 days. In contrast, the affected children from family 6 exhibited a less severe phenotype, which was interpreted as intermediate between mild NLS and a very severe expression of nonlethal PSAT1 deficiency. One child died at age 6 years and the other one was still alive at age 4 years. All affected individuals showed significant IUGR (−1.7 SD to −4.6 SD for term or near-term newborns).

All patients examined showed typical craniofacial abnormalities with microcephaly (−3.4 SD to −6.1 SD for term or near-term newborns), sloping forehead, and micrognathia. A round gaping mouth, low-set and malformed ears, and a short neck were recorded in a large majority of cases. More severe craniofacial abnormalities with frank ocular proptosis, everted lips, and ectropium were less frequent

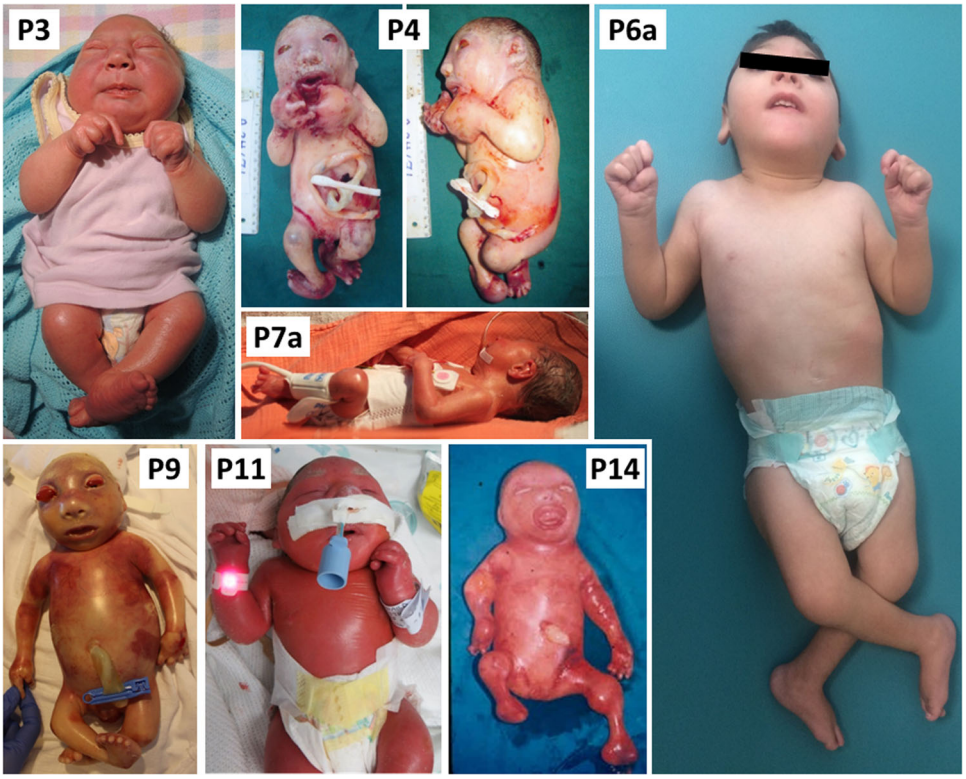

FIGURE 2 Clinical photographs documenting the phenotype in selected cases of this cohort. Variable clinical presentation of several patients as newborns (P3, P4, P7, P9, P11, P14) and at the age of 3.5 years (P6a). Patient ID is indicated on the top of the respective photos

and appeared to correlate with the degree of microcephaly and skin involvement (Tables 1 and S4).

Structural CNS abnormalities were evaluated by ultrasound, MRI, or at autopsy. Abnormal gyration was found in 9 of 11 cases and variable in expression ranging from lissencephaly to pachygyria and polymicrogyria. Cerebellar hypoplasia, hypoplasia/agenesis of the corpus callosum, and hydrocephalus/enlarged ventricles were identified in almost all cases who had detailed examination. Occasionally observed CNS histopathological findings included widespread gliosis, hypoplastic corticospinal tracts, reduced number of anterior horn motor neurons, dysplastic thalami, and abnormal brain vessels.

All affected fetuses and infants had some degree of joint contractures, often with a typical pattern of hands and feet posture (Figure 2). Swelling or edema of hands and/or feet occurred in 10 of 18 patients (56%). Ichthyosis was recorded in all but two cases and was variable in expression. Seven affected individuals showed restrictive dermopathy (39%). Genitourinary abnormalities were reported in 11 of our patients, mostly small kidneys and hypoplastic genitalia. Cleft palate was present in five patients; cataracts were recorded in eight cases. Occasional abnormalities that were recorded included heart defects (patients 9, 12, and 13), sacral agenesis (patient 9), duodenal atresia (patient 7a), underdevelopment of muscles (patient 14), microcornea (patient 12), retinal detachment (patient 10), and preaxial hexadactyly (patient 7a; Tables 2 and S4).

4 | DISCUSSION

The present cohort (15 unrelated families, 14 of them with NLS and one with a very severe form of PSAT1 deficiency) increases the number of molecularly characterized unrelated families affected by NLS to a total of 35 (Acuna-Hidalgo et al., 2014; Bourque et al., 2019; El-Hattab et al., 2016; Mattos et al., 2015; Shaheen et al., 2014), among which a PSAT1 defect turned out to be the most common cause (accounting for 18 out of 35 unrelated cases; 51%), closely followed by PHGDH (16/35; 46%). This distribution may be affected by population selection, as in the present and previously published NLS cases, origin from the Middle East has been dominant with at least one obvious PSAT1 founder allele, c.296C>T, p.(Ala99Val). This particular missense change has been identified in four unrelated families of our cohort and was previously reported in another five unrelated families (Acuna-Hidalgo et al., 2014; El-Hattab et al., 2016). Notably, *PHGDH* variations are more prevalent in nonlethal serine deficiency disorders (Figure 1 and Tables S1–S3). For *PSPH*, there is still only one NLS family (3%) with a presumed causative variant (Acuna-Hidalgo et al., 2014). Therefore, this gene is still awaiting confirmation of its association with NLS. As we could solve the underlying genetic defect in one of the two families previously reported as negative for mutations in the three serine biosynthesis pathway genes (family 4, previously reported as family 11 by Acuna-Hidalgo et al., 2014), there is only one remaining family from that previous study with a clinical diagnosis of NLS and an unidentified genetic

cause. As the phenotype in that family had not a final diagnosis of NLS (according to a careful review of the clinical phenotype), the hypothesis of possible further genetic heterogeneity of NLS (Acuna-Hidalgo et al., 2014) cannot be further supported. In patient 11, we were able to identify only the disease-causing variant on one allele, but the presence of the recurrent NLS-associated *PSAT1* variant (c.296C>T) in the heterozygous state is strongly suggestive that this is another case of PSAT1-associated NLS. The poor quality and quantity of the patient's DNA extracted from the formalin-fixed tissue slides and unavailability of a paternal DNA sample prohibited a full screening for the paternal variant.

The discovery that genes encoding enzymes of the L-serine biosynthesis pathway are mutated in NLS suggested that this phenotype represents the severe end of the spectrum of serine deficiency disorders (Acuna-Hidalgo et al., 2014; Shaheen et al., 2014). However, the proposed more severe impact on the enzymatic activity of NLS-associated changes has not yet been proven. It is known that serine plays a vital role in the cellular proliferation and in development of the CNS and other organs. De novo biosynthesis of L-serine from glycolytic intermediate 3-phosphoglycerate is the essential source of L-serine in mammals (Furuya, 2008). The nonlethal types of serine biosynthesis defects share with NLS a prominent impact on brain development and function, but the degrees of microcephaly and brain dysfunction are extremely variable. For a few PHGDH mutants associated with nonlethal types of serine biosynthesis defects, a considerable residual enzymatic activity in the range of 12–35% of normal has been shown (Klomp et al., 2000; Pind et al., 2002; Tabatabaie et al., 2011).

The present cohort of 14 novel NLS families further expands the spectrum of NLS-associated *PSAT1* and *PHGDH* variants (7 and 3 novel variants, respectively), and genotype analysis confirmed the nonoverlap with genetic changes causing nonlethal serine biosynthesis defects (Figure 1 and Tables S1 and S2). Our observation of the family 6 (intermediate phenotype between a mild form of NLS and very severe expression of nonlethal serine biosynthesis) defect further supports the view that there is a continuum of phenotypic expression from the most severe prenatally lethal forms (patients 12 and 14), moderate NLS expression allowing a short period of postnatal survival (patients 3 and 7a) to a very severe nonlethal type of serine deficiency (patients 6a and 6b). The severity of the disease did not correlate with the identity of the mutated gene. Less severe expression of NLS was instead observed with variants in either *PHGDH* (e.g., patient 3 with the homozygous mutation c.638C>T) or *PSAT1* (e.g., patient 7a with the compound heterozygous changes c.181C>T and c.296C>T). Biallelic nonsense, frameshift or splice site changes, predicting complete loss-of-function of *PSAT1* or *PHGDH* gene products, have never been reported in nonlethal serine biosynthesis disorders and in only one case of NLS (Mattos et al., 2015; Tables S1 and S2). Notably, this affected fetus had a most severe expression of NLS. We report here a homozygous splice acceptor change (c.870-1G>T) in the *PSAT1* gene in patient 14 with a very severe expression of NLS (Figure 2). Notably, the same splice site change was also found in compound heterozygosity with two different missense variants (p.Ala99Val and p.Glu155Gln) in two liveborn patients of our cohort (patients 10 and 13). These findings further support the view that a complete or near-complete loss of enzymatic activity leads to the most severe expression of NLS. Such cases are presumed to end with early fetal loss and are likely to remain undiagnosed. Consistently, there is one report on a homozygous truncating *PHGDH* variant, c.1030C>T, p.(Arg344*), in a case of fetal loss due to nonimmune hydrops fetalis (Monies et al., 2019). In the presence of one allele with a variant causing complete loss of function, the severity of expression appears to be dictated by the nature of the change on the second allele. The phenotypic expression in such cases and in those with homozygous variants allows a preliminary empirical classification of *PSAT1* and *PHGDH* variants according to their impact on protein (enzymatic) function (Tables S1 and S2). Notably, the *PSAT1* allele c.296C>T, p.(Ala99Val), has repeatedly been observed with a somewhat milder expression of NLS, when this change was in the homozygous or in a compound heterozygous state (Acuna-Hidalgo et al., 2014). Consistently, the same homozygous variant has recently been reported in a patient that died at 9 weeks of life and was classified as an intermediate phenotype between NLS and infantile serine biosynthesis defect (El-Hattab et al., 2016). Comparison of all cases with the recurrent *PSAT1* missense change p.Ala99Val in a homozygous state also shows some variability, but the phenotypic spectrum appears to be shifted toward a milder expression (all cases were liveborn at term or near term and were lacking frank proptosis or restrictive dermopathy). Accordingly, the *PSAT1* variant c.129T>G, p.(Ser43Arg), we identified in family 6 with the intermediate clinical expression has been published before with a similar phenotype of serine biosynthesis deficiency with severe prenatal onset microcephaly (Brassier et al., 2016). As it seems that gradual differences in residual enzymatic activity may have great influence on the phenotype, we anticipate quite tight genotype–phenotype correlations, but many more observations are necessary to corroborate the variant-specific phenotype associations. Additional unidentified genetic modifiers or nongenetic factors may also play a role in determining the severity of expression. These considerations underscore the need for functional studies to assess the residual enzymatic activity of mutants associated with variable expressions of serine biosynthesis defects. In fact, a very recently published study using a quantitative, yeast-based growth assay could demonstrate differences in the functional impact of PSAT1 variants, which were in agreement with phenotype annotations. For example, normalized growth estimates were lowest for some NLS-associated variants, such as p.Ser179Leu and p.Cys245Arg (<0.10), intermediate for the variant p.Ala99Val (0.60) associated with a less severe expression of NLS, and mildly impaired for p.Ser43Arg (0.75) reported with a severe form of nonlethal PSAT1 deficiency. The highest level, still significantly below the normal, was calculated for p.Asp100Ala, a variant known to be associated with nonlethal PSAT1 deficiency (Sirr et al., 2020).

When comparing the genotypes observed in serine deficiency disorders and NLS, we noticed that the variants were distributed along the *PHGDH* gene (Figure 1a), but interestingly more than half of

the variants identified with nonlethal PHGDH deficiency disorder resided in the regulatory domain of PHGDH. In contrast, the majority of NLS-associated variants were located in the nucleotide binding domain of PHGDH (Figure 1a). It was previously suggested that alteration of the C-terminal domain of mammalian PHGDH had less impact on the enzymatic activity (Klomp et al., 2000). It was also noted that the presumed regulatory domain had poor conservation across species (Achouri, Rider, Schaftingen, & Robbi, 1997). In the latter study, it was shown that the removal of the carboxyl-terminal 209 amino acids from the rat enzyme lowered but did not abolish the enzyme activity (Achouri et al., 1997). However, differential effects of variants in those genes are not strictly related to the affected protein domains, they may also occur within the same domain, even with mutations at closely neighboring codons. This is exemplified by *PSAT1* mutations p.Asp100Ala and p.Ala99Val, which are known to cause nonlethal PSAT1 deficiency and NLS, respectively (Figures 1b and S3).

In conclusion, this study and a literature review show that pathogenic *PHGDH* and *PSAT1* variants lead to a spectrum of human disorders and that NLS per se has varying degrees of phenotypic expression likely representing the extreme end of a continuum. We postulate that the individual residual enzyme activity of mutant proteins is the major determinant of the phenotypic variability, but other genetic and nongenetic modifiers cannot be excluded. Further functional studies and modeling of PHGDH and PSAT1 defects are needed to explore in detail the functional basis of the phenotypic variability.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. All variants observed in the present cohort were submitted to the genetic Leiden Open Variation Database LOVD (https://databases.lovd.nl/) and the respective accession numbers are mentioned in Table 2.

ORCID

Eva Bermejo-Sánchez https://orcid.org/0000-0001-7282-2714
Isolina Riaño-Galán https://orcid.org/0000-0003-2125-5101
Marie-Laure Vuillaume http://orcid.org/0000-0003-1080-972X
Martin Zenker http://orcid.org/0000-0003-1618-9269

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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