Supporting Information

regarding the original article

Expanding the genotypic and phenotypic spectrum of severe serine biosynthesis disorders

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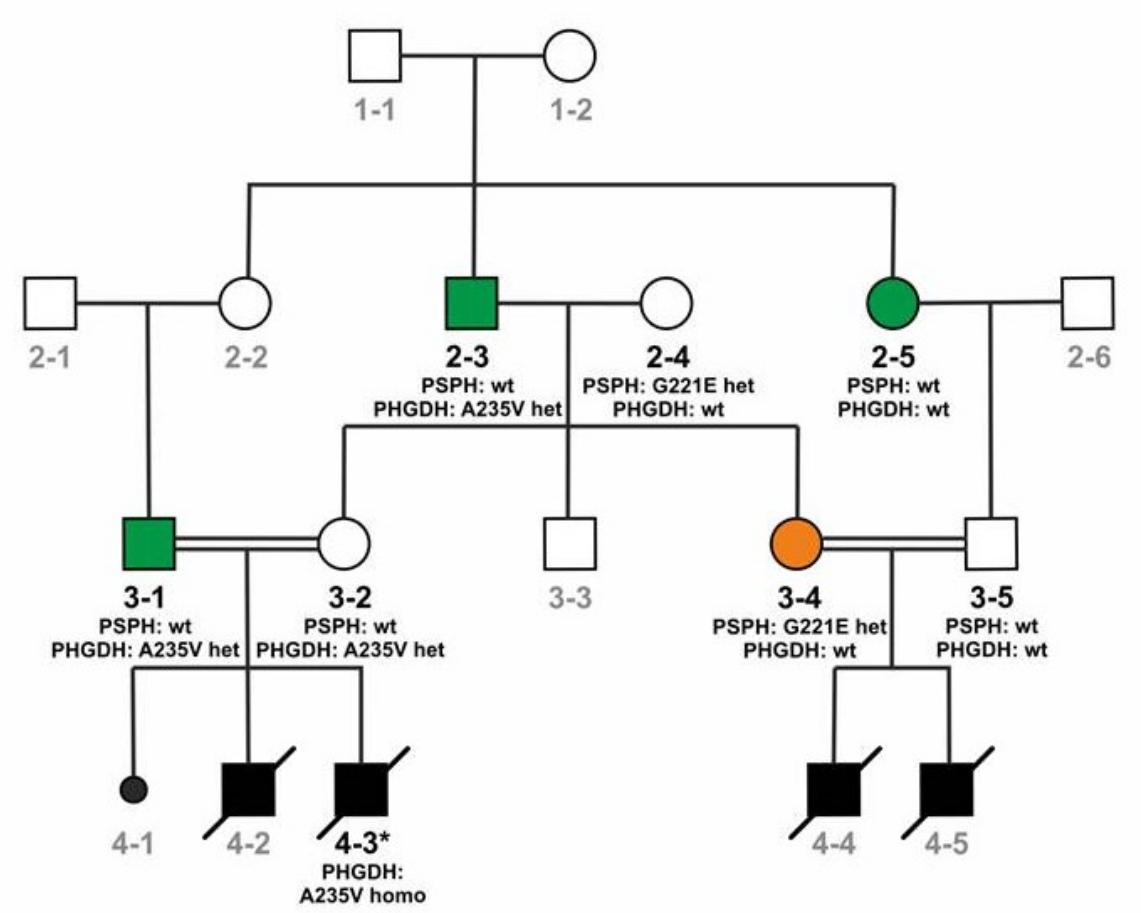
Supporting Figure S1: Pedigree and molecular testing history in family 4

Family 4, previously published as family 11 by Acuna-Hidalgo et al. (Acuna-Hidalgo et al., 2014), is a multi-consanguineous family that was initially considered as affected with Neu-Laxova syndrome (NLS) in two branches of the family (**Supp. Figure S1**). All affected individuals were stillborn or neonatal deaths. Only from one of them, FFPE tissue material was available for DNA extraction, but the material was insufficient in quality and amount to allow a broader mutation screening. The typical NLS phenotype was well documented in this individual (**Figure 2**, P4). Initial whole exome sequencing (WES) was performed in 2012 on one individual who was considered as an obligate carrier for the familial disease (**Supp. Figure S1**). In this individual a heterozygous variant, c.662G>A (p.Gly221Glu), of the *PSPH* gene was identified, but this variant was not considered to be causative for the disease on the basis of linkage and segregation data. The family remained unsolved and the possibility of further genetic heterogeneity of NLS was discussed (Acuna-Hidalgo et al., 2014). Later on, WES in three additional family members identified a likely disease-causing *PHGDH* variant in one branch of the family, but not in the other. Reappraisal of the family history showed that the two affected offspring in the branch of the family without the *PHGDH* variant were born after 8 months and 33 weeks of gestation, respectively, and both died immediately after birth. The family reported skin abnormalities and considered the disorder as similar to what occurred in the other branch of the family, but there was very limited documented information regarding the deceased newborns, no photos, no measurements, no autopsy. Thus with the new results, it has to be assumed that the disease in that branch of the family did not have the same genetic cause as in the one where we could demonstrate the *PHGDH* variant segregating with a well documented NLS phenotype. It is likely that the other lethal condition in the family was not even NLS.



Supporting Figure S1: The pedigree of family 4, previously published as family 11 by Acuna-Hidalgo et al. (Acuna-Hidalgo et al., 2014), is depicted. Materials for genetic testing were available from individuals whose ID numbers are printed in black, while no material was available from the others (ID numbers in grey). From individual 4-3 only FFPE tissue material was available (*) allowing the extraction of very poor DNA. Initial whole exome sequencing (WES) was performed on individual 3-4 (orange circle) who was considered as an obligate carrier for the presumed familial mutation responsible for the disease. The heterozygous *PSPH* variant, c.662G>A (p.Gly221Glu; G221E), which was identified in this person, was inherited from her mother (2-4) but not present in any of the other family members who were expected to be carriers of the disease (assuming autosomal recessive inheritance). In a second approach, we now performed WES on three additional family members considered to be obligate carriers (green boxes and circles). This analysis revealed a heterozygous variant in the *PHGDH* gene in individuals 2-3 and 3-1, while 2-5 was wild type (wt) for this variant. Additional targeted Sanger sequencing also confirmed the carrier status for this variant in 3-2 and homozygosity in the affected fetus 4-3, while the variant was not present in the other branch of the family (right).

Supporting Results and Figures S2-S3

Structural analysis and molecular modeling

To provide a better understanding of the functional effects of observed missense variants in *PHGDH* and *PSAT1*, structural analysis was performed based on the crystal structures of PHGDH (PDB code: 2G76) and PSAT1 (PDB code: 3E77). Wildtype and mutant residues were modeled with SwissModel (Guex & Peitsch, 1997) and RasMol (Sayle & Milner-White, 1995) was used for structure analysis and visualization.

Structural information is available for the N-terminal parts of the two proteins. PHGDH: amino acids 6-307 (of 533); PSAT1: amino acids 17-370 (of 496). No structure is available for the C-terminal regulatory domain of eukaryotic PHGDHs.

Results

For PHGDH, the novel variants observed in the present study, p.Thr213Met (T213M) and p.Ala235Val (A235V), were particularly analyzed. Both variants are located in the immediate vicinity of the NAD+ cofactor (**Supp. Figure S2A**). A closer inspection of the interactions revealed that both T213 (**Supp. Figure S2B**) and A235 (**Supp. Figure S2D**) form direct interactions with NAD+ thereby stabilizing cofactor binding. At both sites, the replacement by bulkier residues results in steric problems ('clashes') that are expected to hamper NAD+ binding (**Supp. Figure S2C,E**) thereby probably causing a reduced enzymatic activity. An inspection of the variants previously reported (**Supp. Table S1**; **Supp. Figure S2A**) reveals that a significant portion of them also cluster around the substrate and cofactor binding site (R54C, S55F, R135W, V261M, E265K, A286P) suggesting that they may act by a similar mechanism, i.e. by causing reduced cofactor or substrate affinity.

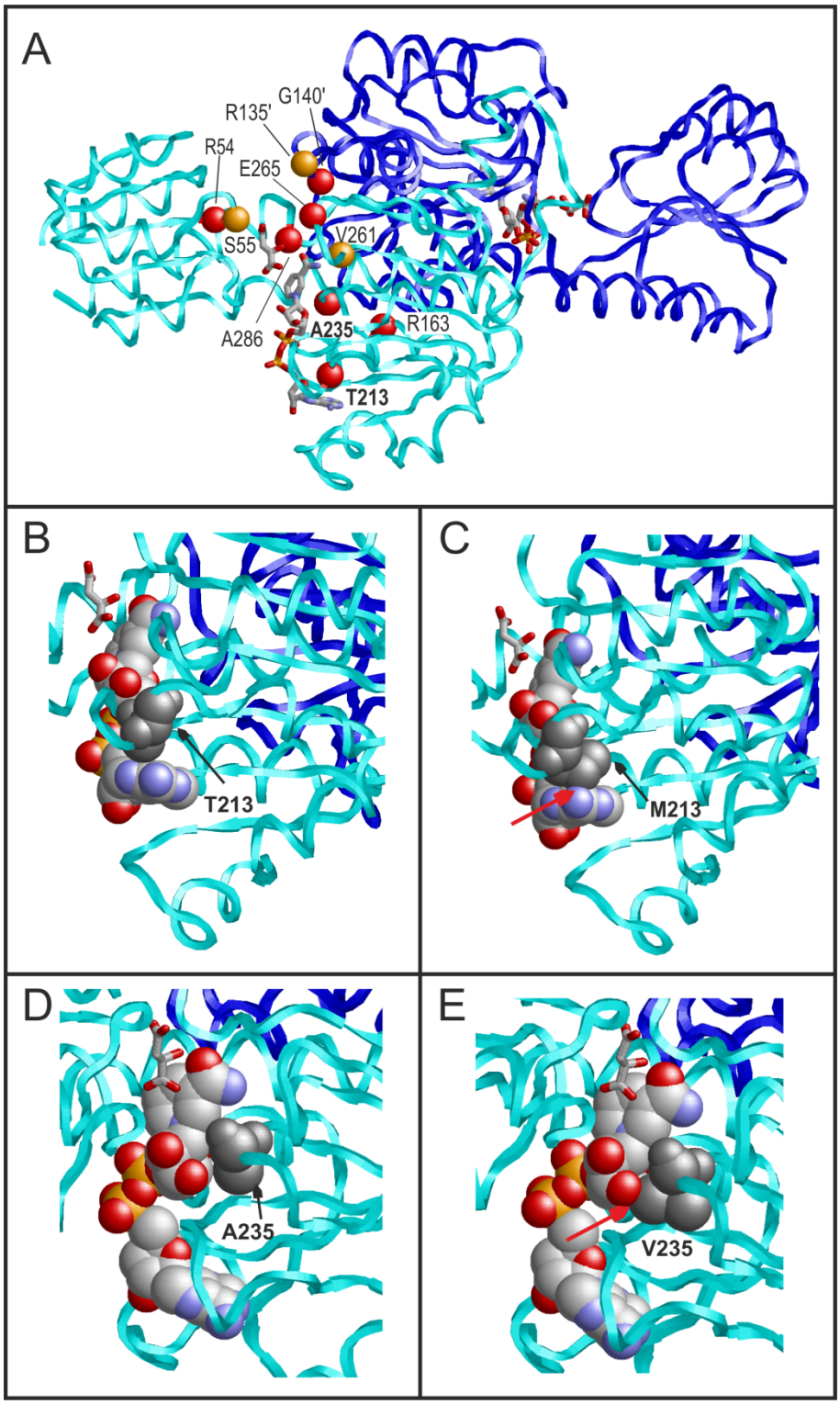
The remaining variants observed in the globular PHGDH nucleotide-binding domain (G140R, R163W, R163Q) are located at the dimer interface (**Supp. Figure S2A**) suggesting that they may primarily affect dimer stability. Importantly, the dimer interface was shown to play a crucial role in the overall stability of this protein family and an intact dimer is essentially required for optimal enzymatic activity (Mishra et al., 2012). Therefore, amino acid side-chain changes that are destabilizing the dimer configuration, are also expected to cause a reduced enzymatic activity.

Compared to PHGDH, the novel missense variants observed in PSAT1, p.Arg61Trp (R61W), p.Gly79Trp (G79W), p.Glu155Gln (E155Q), and p.Cys245Arg (C245R), are rather distributed over the structure (**Supp. Figure S3A**). Two of the variants (G79W, E155Q) are located in the vicinity of the pyridoxal 5'-phosphate (PLP) cofactor binding site. A closer inspection of the structure reveals that G79 directly interacts with PLP (**Supp. Figure S3B**) and that the exchange to a bulky tryptophan causes steric clashes with the PLP and with adjacent amino acids (**Supp. Figure S3C**). From the structural analysis, the E155Q exchange is also expected to affect PLP binding, although by a slightly different mechanism. In the wildtype, E155 forms two hydrogen bonds to the protein backbone thereby stabilizing the orientation of W107 that exhibits tight stacking interactions with the PLP (**Supp. Figure S3D**). The different physico-chemical properties of the Q155 sidechain result in the loss of one hydrogen bond which is expected to destabilize the W107 position and consequently its interaction with PLP (**Supp. Figure S3E**). In addition to the two variants analyzed above, the previously reported S179L variant **(Supp. Table S2**; **Supp. Figure S3A**) is also located close to the PLP binding site suggesting similar unfavorable effects on cofactor binding. Interestingly, similar

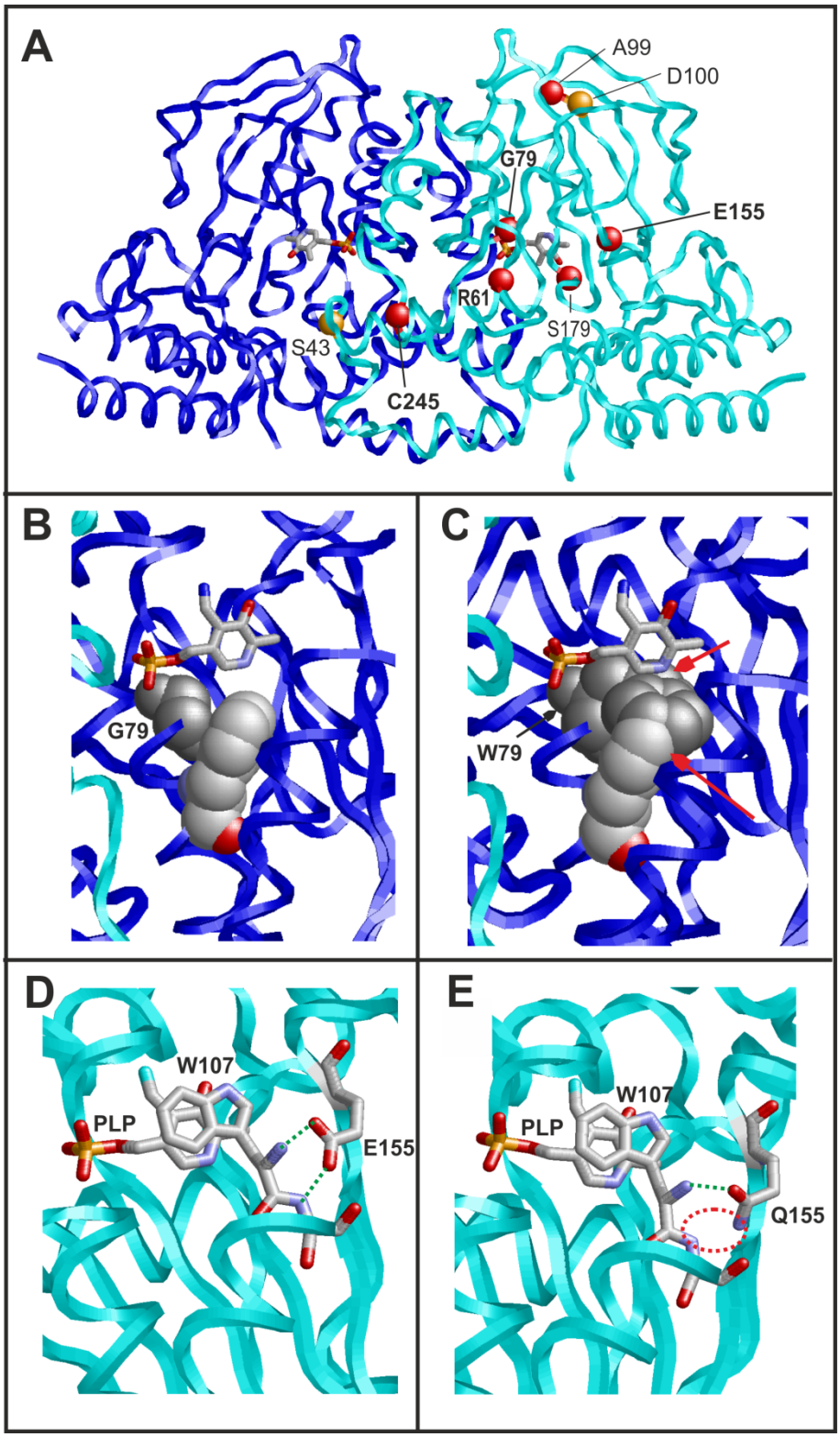
structural effects on cofactor binding have also been observed for the T213M and A235V variants in PHGDH (**Supp. Figure S2**).

C245 is located close to the PSAT1 dimer interface (**Supp. Figure S3F**) and a replacement by arginine results in steric clashes with V39 and M42 (**Supp. Figure S3G**), which probably reduce dimer stability. The region of these clashes is close to the site of the S43R variant (**Supp. Figure S3A**), which has been previously described as disease-related and was also found in this study (**Supp. Table S2**). Since previous investigations have shown that the dimeric configuration of the enzyme is essential for function (John, 1995; Mishra, Ali, Nozaki, & Bhakuni, 2010), variants that destabilize the dimer are expected to cause a reduced enzymatic activity.

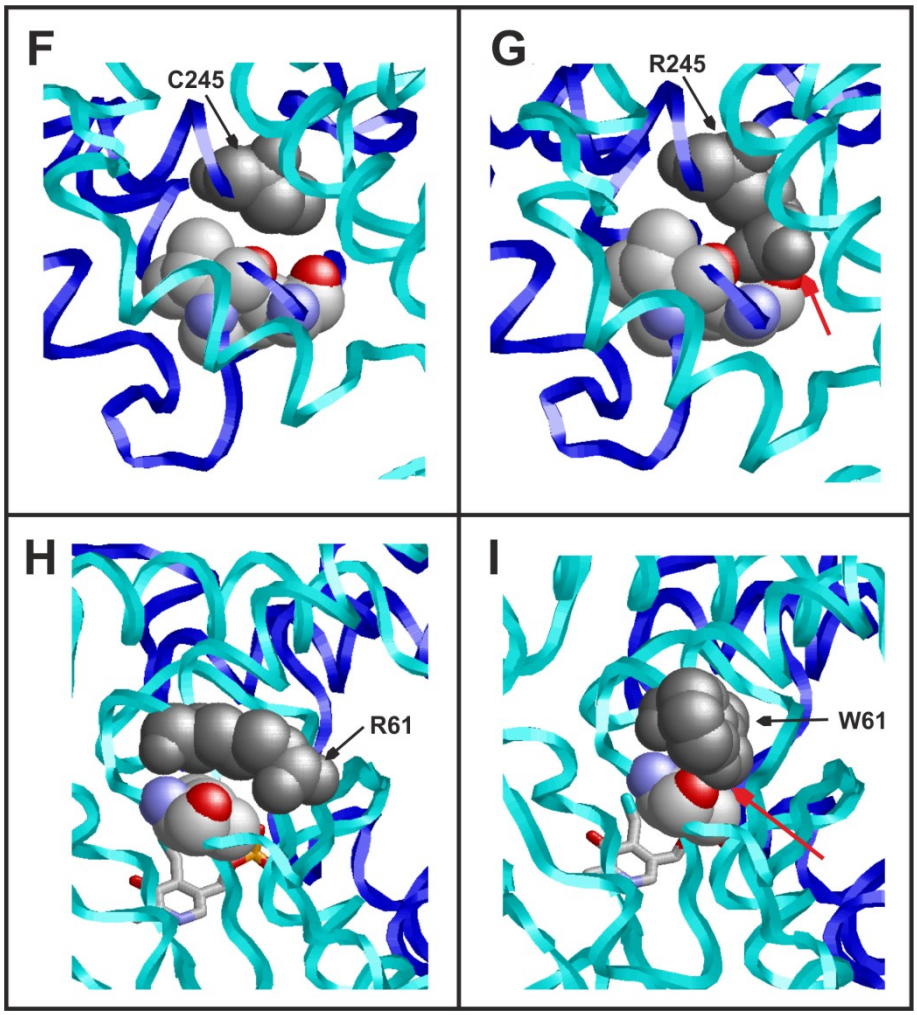
Like the previously described A99V and D100A variants, the newly discovered R61W variant is neither located close to the dimer interface nor to the substrate/cofactor binding site (**Supp. Figure S3A**) suggesting that these exchanges mainly affect protein/domain stability itself. Molecular modeling indicates that R61W causes a destabilization of the enzyme fold by steric clashes (R61W; **Supp. Figure S3I**)



Supporting Figure S2: Structural effects of sequence variants in PHGDH. (**A**) Overview of the dimeric PHGDH structure (cyan, blue) indicating the position of the missense variants. The bound malate and NAD+ are shown in stick presentation and colored according to the atom type (cpk coloring). In one subunit, NLS- and SBDNL-associated variants are highlighted by red and orange balls, respectively. Novel variants detected in the present study are labelled in bold and their structural effect is shown in detail in the following panels. (**B**) T213 (dark grey) forms favorable interactions with NAD+ (space-filled; cpk coloring), whereas (**C**) M213 forms steric clashes (red arrow) with NAD+. (**D**) A235 (dark grey) forms favorable interactions with NAD+ (cpk coloring), whereas (**E**) V235 forms steric clashes (red arrow) with NAD+.



Supporting Figure S3A-E (continued on following page)



Supporting Figure S3F-I

Supporting Figure S3: Structural effects of sequence variants in PSAT1. (**A**) Overview of the dimeric PSAT1 structure (cyan, blue) indicating the position of the missense variants. The bound pyridoxal 5'-phosphate (PLP) is shown in stick presentation and colored according to the atom type (cpk coloring). In one subunit, NLS- and SBDNL-associated variants are highlighted by red and orange balls, respectively. Novel variants detected in the present study are labelled in bold and their structural effect is shown in detail in the following panels. (**B**) G79 (dark grey) forms favorable interactions with PLP (stick presentation; cpk coloring), whereas (**C**) W79 forms steric clashes (red arrows) with PLP and the adjacent F83 (space-filled presentation; cpk coloring). (**D**) E155 forms two backbone hydrogen bonds (green dotted lines) that stabilize the interaction of W107 with PLP. (**E**) Q155 can only form one hydrogen bond resulting in a destabilization of the W107 segment (red dotted circle) and a weakened interaction with PLP. Residues 100-106 have been omitted for clarity in this presentation. (**F**) C245 (dark grey) is located close to the dimer interface in the vicinity of V39/M42 (cpk coloring). (**G**) R245 forms clashes with V39/M42 (red arrow) thereby destabilizing the dimer interface. (**H**) R61 (dark grey) is located close to V66 (cpk coloring) but does not form any clashes. (**I**) The bulkier sidechain of W61 causes steric clashes with V66 (red arrow) resulting in a domain destabilization.

Supporting Tables S1-S3

The following three tables provide an overview on reported variants in Neu-Laxova syndrome (NLS) and non-lethal types of serine biosynthesis defects (SBDNL). Novel variants that are first reported in a scientific article here are printed in bold and all variants observed in the present cohort (comprising 15 unrelated families) are highlighted in green.

Interpretation of variants as NLS-associated or SBDNL-associated change was primarily based on the reported phenotype in homozygous individuals. For variants that have not been observed in a homozygous constellation, thus far, the interpretation was as follows: (i) Frameshift variants and one multi-exon deletion of *PHGDH*, which have only been observed in compound heterozygosity with a non-truncating variant and not in the homozygous state were classified as probable LoF (loss-of-function) alleles. (ii) Variants that have been observed in an individual with SBDNL in compound heterozygosity with a variant classified as NLS-associated variant or probable LoF allele were classified as probable SBDNL-associated variant, because it was evident that this allele confers the less severe phenotype (presumably higher residual enzyme activity). (iii) Non-truncating variants that have been observed in an individual with NLS in compound heterozygosity with a variant classified as NLS-associated variant or probable LoF allele were classified as probable NLS-associated variant, because it was evident that this allele was not able to ameliorate the phenotype towards a less severe non-lethal condition. (iv) Two non-truncating (e.g. missense), not otherwise classified variants observed in compound heterozygosity in association with a NLS phenotype were both classified as possible NLS-associated variants, because it may be assumed that neither of the two variants was able to ameliorate the phenotype towards a less severe non-lethal condition.

Variants refer to the reference sequences of *PHGDH* (NM_006623.4; NG_009188.1), *PSAT1* (NM_058179.4; NG_012165.1) and PSPH (NM_004577.4). Compliance with HGVS nomenclature was verified using the Mutalyzer online software tool (https://mutalyzer.nl/).

Further legends are provided for each table separately.

Supporting Table S1: *PHGDH* variants and associated phenotypes

Variant	Consequence on RNA /protein	Homozygous phenotype	Reference	Comp. het. phenotype	Second allele(s)	Reference	Interpretation
c.1A>C	p.?	NLS	(Bourque et al., 2019)				NLS-associated variant
c.138+2dup	p.? ¶	NO		SBDNL	c.1129G>A	(Benke et al., 2017)	Not classified
c.160C>T	p.(Arg54Cys)	NLS	This paper	NLS	Exon del	(Acuna-Hidalgo et al., 2014)	NLS-associated variant
c.164C>T	p.(Ser55Phe)	NO		SBDNL	c.1429dup	(Poli et al., 2017)	Probable SBDNL-associated variant
c.403C>T	p.(Arg135Trp)	NO		SBDNL	c.487C>T	(Brassier et al., 2016)	Probable SBDNL-associated variant
					c.712delG	(Tabatabaie et al., 2009)	
c.418G>A	p.(Gly140Arg)	NLS	(El-Hattab et al., 2016; Shaheen et al., 2014)	NO			NLS-associated variant
c.487C>T	p.(Arg163Trp)	NO		SBDNL	c.403C>T	(Brassier et al., 2016)	Not classified
c.488G>A	p.(Arg163Gln)	NLS	(Shaheen et al., 2014; this paper)	NO			NLS-associated variant
c.638C>T	**p.(Thr213Met)**	NLS	This paper	NO			NLS-associated variant
c.704C>T	**p.(Ala235Val)**	NLS	This paper	NO			NLS-associated variant
c.714del †	p.(Ile239Serfs*69)	NO		SBDNL	c.403C>T	(Tabatabaie et al., 2009)	Probable LoF allele
c.746T>C	p.(Leu249Pro)	NLS	(Cavole et al., 2020)	NO			NLS-associated variant
c.781G>A	p.(Val261Met)	SBDNL	(Coskun et al., 2009; Tabatabaie et al., 2009)	NO			SBDNL-associated variant
c.793G>A	p.(Glu265Lys)	NLS	(Acuna-Hidalgo et al., 2014)	NO			NLS-associated variant
c.856G>C	p.(Ala286Pro)	NLS	(Acuna-Hidalgo et al., 2014)	NO			NLS-associated variant
c.1030C>T	p.(Arg344*)	NIHF ‡	(Monies et al., 2019)	NO			Probable LoF allele
c.1117G>A	p.(Ala373Thr)	SBDNL	(Tabatabaie et al., 2009)	NO			SBDNL-associated variant
c.1129G>A	p.(Gly377Ser)	SBDNL	(Tabatabaie et al., 2009)	SBDNL	c.138+2dup	(Benke et al., 2017)	SBDNL-associated variant
c.1263C>G	p.(Cys421Trp)	NO		NLS	c.1468G>A	(Ni et al., 2019)	Probable NLS-associated variant
c.1273G>A	p.(Val425Met)	SBDNL	(Klomp et al., 2000; Kraoua et al., 2013)	SBDNL	c.1471C>T	(Meneret et al., 2012)	SBDNL-associated variant
c.1286G>T	p.(Gly429Val)	SBDNL/NLS	(Benke et al., 2017; El-Hattab et al., 2016)	NO			Intermediate variant
c.1297C>T	p.(Gln433*)	NLS	(Mattos et al., 2015)	NO			NLS-associated variant
c.1429dup	p.(Met477Asnfs*51)	NO		SBDNL	c.164 C>T	(Poli et al., 2017)	Probable LoF allele
c.1468G>A	p.(Val490Met)	SBDNL	(Klomp et al., 2000; Pind et al., 2002)	NLS	c.1263C>G	(Ni et al., 2019)	SBDNL-associated variant / Intermediate variant
c.1471C>T	p.(Arg491Trp)	NO		SBDNL	c.1273G>A	(Meneret et al., 2012)	Not classified

Del. exons 10-12	p.?	NO		NLS	c.160C>T	(Acuna-Hidalgo et al., 2014)	Probable LoF allele

Comp. het., compound heterozygous; Del., deletion; LoF, loss-of-function.

Phenotype: NIHF, non-immune hydrops fetalis; NLS, Neu-Laxova syndrome; NO, not observed / not reported; SBDNL, serine biosynthesis defect, non-lethal .

† reported in the literature as c.712delG. ¶ Variant is assumed to affect splicing.

‡ only rudimentary clinical information provided for this case reported with non-immune hydrops fetalis.

Variant observed in the present cohort. **Novel variant.**

Supporting Table S2: *PSAT1* variants and associated phenotypes

Variant	Consequence on RNA /protein	Homozygous phenotype	Reference	Comp. het. phenotype	Second allele(s)	Reference	Interpretation
c.1A>G	**p.?**	NLS	This paper	NO			NLS-associated variant
c.44C>T	p.(Ala15Val)	NO		SBDNL	c.432delA	(Glinton et al., 2018)	Probable SBDNL-associated variant
c.107del	p.(Gly36Alafs*7)	NO		SBDNL	c.299A>C	(Hart et al., 2007)	Probable LoF allele
c.129T>G	p.(Ser43Arg)	SBDNL	(Brassier et al., 2016; this paper)	NO			SBDNL-associated variant
c.181C>T	**p.(Arg61Trp)**	NO		NLS	c.296C>T	This paper	Probable NLS-associated variant
c.208T>A	p.(Tyr70Asn)	NO		NLS	c.1024C>T	(Ni et al., 2019)	Possible NLS-associated variant
c.233G>C	p.(Gly78Ala)	MCA ‡	(Monies et al., 2017)	NO			Not classified ‡
c.235G>T	**p.(Gly79Trp)**	NLS	This paper	NO			NLS-associated variant
c.296C>T	p.(Ala99Val)	NLS	(Acuna-Hidalgo et al., 2014; El-Hattab et al., 2016; this paper)	NLS	c.536C>T	(Acuna-Hidalgo et al., 2014)	NLS-associated variant (common allele)
					c.181C>T	This paper	
					c.870-1G>T	This paper	
					Missing allele	This paper	
c.299A>C	p.(Asp100Ala)	NO		SBDNL	c.107del	(Hart et al., 2007)	SBDNL-associated variant
c.432del	p.(Asp145Metfs*49)	NO		SBDNL	c.44C>T	(Glinton et al., 2018)	Probable LoF allele
c.463G>C	**p.(Glu155Gln)**	NO		NLS	c.870-1G>T	This paper	Probable NLS-associated variant
c.536C>T	p.(Ser179Leu)	NLS	(Acuna-Hidalgo et al., 2014)	NLS	c.296C>T	(Acuna-Hidalgo et al., 2014)	NLS-associated variant
c.733T>C	**p.(Cys245Arg)**	NLS	This paper	NO			NLS-associated variant
c.870-1G>T	**p.?** ¶	NLS	This paper	NLS	c.296C>T	This paper	NLS-associated variant
					c.463G>C	This paper	
c.955del	**p.(Arg319Aspfs*14)**	NLS	This paper	NO			NLS-associated variant
c.1023_1027 delinsAGACCT	p.(Arg342Aspfs*6)	NLS	(Acuna-Hidalgo et al., 2014)	NO			NLS-associated variant
c.1024C>T	p.(Arg342Trp)	NO		NLS	c.208T>A	(Ni et al., 2019)	Possible NLS-associated variant

Comp. het., compound heterozygous; LoF, loss-of-function.
Phenotype: MCA, multiple congenital anomalies; NLS, Neu-Laxova syndrome; NO, not observed / not reported; SBDNL, serine biosynthesis defect, non-lethal.
‡ only rudimentary clinical information provided for this case; therefore no interpretation is provided. ¶ Variant is assumed to affect splicing.
Variant observed in the present cohort. **Novel variant.**

Supporting Table S3: *PSPH* variants and associated phenotypes

Variant	Consequence on RNA /protein	Homozygous phenotype	Reference	Comp. het. phenotype	Second allele(s)	Reference	Interpretation
c.94G>A	p.(Asp32Asn)	NO		SBDNL	c.155T>C	(Veiga-da-Cunha et al., 2004)	Not classified
c.103G>A	p.(Ala35Thr)	SBDNL	(Vincent et al., 2015)	SBDNL	NO		SBDNL-associated variant
c.131T>G	p.(Val44Gly)	NO		SBDNL	c.421G>A	(Byers et al., 2016)	Not classified
c.155T>C	p.(Met52Thr)	NO		SBDNL	c.94G>A	(Veiga-da-Cunha et al., 2004)	Not classified
c.267del	p.(Gly90Alafs*2)	NLS	(Acuna-Hidalgo et al., 2014)	NO			NLS-associated variant
c.421G>A	p.(Gly141Ser)	NO		SBDNL	c.131T>G	(Byers et al., 2016)	Not classified

Comp. het., compound heterozygous.

Phenotype: NLS, Neu-Laxova syndrome; NO, not observed / not reported; SBDNL, serine biosynthesis defect, non-lethal .

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