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Research Paper

Impact of genetic and non-genetic factors on phenotypic diversity in NBAS-associated disease

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ABSTRACT

Biallelic pathogenic variants in neuroblastoma-amplified sequence (*NBAS*) cause a pleiotropic multisystem disorder. Three clinical subgroups have been defined correlating with the localisation of pathogenic variants in the *NBAS* gene: variants affecting the C-terminal region of NBAS result in SOPH syndrome (short stature, optic

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atrophy, Pelger-Huët anomaly), variants affecting the Sec 39 domain are associated with infantile liver failure syndrome type 2 (ILFS2) and variants affecting the ß-propeller domain give rise to a combined phenotype. However, there is still unexplained phenotypic diversity across the three subgroups, challenging the current concept of genotype-phenotype correlations in NBAS-associated disease. Therefore, besides examining the genetic influence, we aim to elucidate the potential impact of pre-symptomatic diagnosis, emergency management and other modifying variables on the clinical phenotype. We investigated genotype-phenotype correlations in individuals sharing the same genotypes ($n = 30$ individuals), and in those sharing the same missense variants with a loss-of-function variant in trans ($n = 38$ individuals). Effects of a pre-symptomatic diagnosis and emergency management on the severity of acute liver failure (ALF) episodes also were analysed, comparing liver function tests (ALAT, ASAT, INR) and mortality. A strong genotype-phenotype correlation was demonstrated in individuals sharing the same genotype; this was especially true for the ILFS2 subgroup. Genotype-phenotype correlation in patients sharing only one missense variant was still high, though at a lower level. Pre-symptomatic diagnosis in combination with an emergency management protocol leads to a trend of reduced severity of ALF. High genetic impact on clinical phenotype in NBAS-associated disease facilitates monitoring and management of affected patients sharing the same genotype. Pre-symptomatic diagnosis and an emergency management protocol do not prevent ALF but may reduce its clinical severity.

1. Introduction

Biallelic pathogenic variants in neuroblastoma-amplified sequence (*NBAS*) cause an autosomal recessive disease with multiple organ system involvement. Symptoms include fever-triggered recurrent acute liver failure (ALF) with onset in infancy, short stature, skeletal dysplasia, immune dysfunction, optic atrophy, and neurological abnormalities, among others. While some affected individuals present with a primarily hepatic phenotype, others show a multisystemic disease without ALF episodes. Within an international, multicentre study including 110 individuals from 97 families, we showed that three clinical subgroups can be identified based on localization of missense variants and in-frame deletions that directly relate to the affected region of the NBAS protein [1]. Missense variants or in-frame deletions affecting the C-terminal region are associated with a multisystemic phenotype named SOPH syndrome (short stature, optic atrophy and Pelger-Huët anomaly; MIM 614800), missense variants or in-frame deletions affecting the Sec39 domain cause a primarily hepatic phenotype characterized by fever-triggered recurrent ALF with onset in infancy (infantile liver failure syndrome type 2, ILFS2; MIM 616483), whereas missense variants or in-frame deletions affecting the β-propeller domain lead to a combined phenotype with a multisystem involvement and ALF [1].

However, this variant-based classification and its possible role for phenotypic prediction has limitations: first, not all affected individuals can be assigned to one of the three subgroups due to a variant outside the above-mentioned domains or compound-heterozygosity for missense variants affecting two different regions of the protein [1]. Second, there are considerable phenotypic differences among individuals belonging to the same subgroup [2]. It seems likely, that other genetic and/or environmental factors contribute to disease presentation. Given the wide heterogeneity of *NBAS* variants, few individuals have the same genotype with the exception of Yakuts (an isolated population living in Russia) sharing homozygous founder variant (c.(5741G > A)) affecting the C-terminal region [3]. To explore the genetic determination of the clinical phenotype in NBAS-associated disease in more detail, we studied genotype-phenotype correlations in individuals sharing the same genotypes. Furthermore, to investigate the hypothesis that the expressed allele determines the phenotype in NBAS-associated disease [1], cases with loss-of-function *NBAS* variants in trans have been considered like cases having only the missense variant and were investigated accordingly.

Besides the genotype, environmental factors such as specific disease management play an important role in the determination of the phenotype. Early antipyretic treatment together with glucose and lipid infusions have been reported to reduce severity of ALF episodes in NBAS-associated disease [4]; its effect, however, has not been studied systematically. To minimize the effect of different genotypes, the impact of an emergency management protocol is best studied in individuals sharing the same genotype. As the impact of such an emergency management protocol should be highest when started early in life, as often seen in families with affected siblings, we hypothesized that emergency management protocol and early diagnosis within families with more than one affected sibling lead to an attenuated course of ALF episodes.

2. Methods

Individuals were recruited within a retrospective, multicentre, observational study. Inclusion criteria were individuals with biallelic pathogenic variants in *NBAS* (NM_015909.3), with at least one further affected individual with the same genotype; and individuals sharing one missense variant with another individual, if the *NBAS* variant in trans was a loss-of-function variant. Individuals were excluded from this study, if there was no valid individualized clinical data available. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2013. Ethical approval was obtained from the institutional ethics committee of the Medical Faculty, University of Heidelberg (S-035/2014). Informed consents were obtained locally from all individuals or their parents (in case of minors). Otherwise, only previously published clinical information was used.

All cases have been either previously published or are followed by one of the co-authors. To search for published cases, PubMed was screened using the mesh terms “NBAS AND/OR SOPH” and “NBAS AND/OR ILFS2” on 15th of January 2022. Each genotype was assigned to one of the three subgroups as previously described [1]. Data collection was finished in June 2022.

Physicians contributing to this study completed a case report form (CRF) for each individual included in this study. In the CRF basic information such as age and sex assigned at birth, the individuals’ genotype and detailed clinical characteristics were assessed. These items were chosen based on expert opinions on clinical characteristics of NBAS-associated disease. The following 17 clinical characteristics named according to the “human phenotype ontology” (HPO) were included: ALF (HP:0006554), continuously elevated liver transaminases (cELT) (HP:0002910), small for gestational age (SGA) (HP:0001518), short stature (HP:0004322), abnormality of the vertebral column (HP:0000925), reduced bone mineral density (HP:0004349), delayed bone age (HP:0002750), delayed closure of fontanels (HP:0001476), neurodevelopmental delay (HP:0012758), optic atrophy (HP:0000648), abnormality of the integument (HP:0001574), reduced natural killer cell (NK-cell) count (HP:0040218), decreased circulating immunoglobulin G (IgG) level (HP:0004315), Pelger-Huët anomaly (PHA) (HP:0011447), muscular hypotonia (HP:0001252), diabetes mellitus (HP: 0000819), facial dysmorphism (HP:0001999) and high pitched voice (HP:0001620). ALF was defined according to the inclusion criteria of the

PALF study [5]. Each characteristic was rated "yes", "no" or "n.a." (not available, if the requested data was not available or not provided). Clinical characteristics were termed "concordant", if they were rated either "yes" or "no" in >75% of individuals per genotype (where data were available), otherwise "discordant".

Age at implementation of an emergency management protocol as well as age at diagnosis and number of ALF episodes, both after implementation of an emergency management protocol as after diagnosis, were assessed. Severity of ALF-crises was quantified comparing maximum activities of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and international normalized ratio (INR) levels during ALF episodes using the one-tailed dependent *t*-test for paired samples and the effect power was measured using Cohen's d for paired samples [6]. Death and liver transplantation due to ALF were also captured. The group difference for lethal outcome was measured with a Boschloo's test. Statistical analysis including creation of figures was computed with Microsoft Excel and R language version 4.2.0.

3. Results

3.1. Cohort description

In total 60 individuals were included. Thirty individuals shared one of 14 different genotypes (genotypes 1–14, Table 1). Thirtyeight individuals shared one of eight missense variants (M 1 – M 8) with a loss-of-function variant in trans (Table 2). Thus, eight individuals sharing the same genotype were included in both study parts (Table 2).

All but three individuals included in this study have been published previously [1,2,4,7–32], for detailed information see Supplementary Table S1. NBAS120 is a novel patient, originating from the United States, and NBAS133 and NBAS134 are siblings of a previously published individual (NBAS18, [4]). For 44 of the 60 individuals, additional clinical data were provided for this study (Supplementary Table S1).

Twenty-nine individuals were male and 31 were female. Median age at last visit was 6.8 years (range 1.3–34.6 years). Forty-eight individuals were alive at the time of reporting, twelve individuals had died at a median age of 3.1 years (range 1.3–34.6 years), and one individual underwent liver transplantation (for further details see Supplementary Table S2). Individuals from all three subgroups were included. Four individuals could not be assigned to any of the three subgroups: two individuals with one missense variant affecting the Sec39 domain and one affecting the β-propeller domain in trans (genotype 4; c.[2674G > T], c.[1018G > C]), and two individuals with both missense variants located between the domains Sec39 and β-propeller (genotype 5; c.[6805G > T], c.[1550G > A]). For a detailed overview of individuals' *NBAS* variants see Tables 1 (individuals sharing the same genotype) and 2 (individuals sharing one missense variant).

3.2. Genotype-phenotype correlation in individuals sharing the same genotype

To analyze genotype-phenotype correlations, we first looked at concordance and discordance rates of clinical characteristics in the individuals sharing the same genotype. For all genotypes, 88.8% of clinical characteristic were concordant, while 11.2% were discordant. Concordance of clinical characteristics was highest in the ILFS2 subgroup (96.7%), while for the SOPH subgroup, concordance rate was 85.1% and for the combined subgroup 77.8% (Fig. 1). Next, we analysed to what extend single clinical characteristics were shared among individuals with the same genotype. Some clinical characteristics were concordant throughout all genotypes (100%), namely delayed closure of fontanels, optic atrophy, muscular hypotonia and high-pitched voice. There was also a very high concordance rate (90% - <100%) for neurodevelopment delay, abnormality of the integument and facial dysmorphism. Furthermore, high concordance rates (80% - < 90%) were found for ALF, SGA, short stature, abnormality of the vertebral column, decreased circulating IgG level, PHA and diabetes mellitus. Only the clinical characteristics cELT, reduced bone mineral density and reduced NK-cell count had concordance rates of <80% (76.9%, 75% and 50.0%, respectively); however, there was a high rate of missing data for reduced

Table 1
NBAS variants (NM_015909.3; NP_056993.2) of all individuals included in this study sharing the same genotype.

Genotype	PID	Region/ domain of NBAS affected	Allele 1 nucleotide change	Allele 1 protein change	Allele 2 nucleotide change	Allele 2 protein change
1	24 25 26	β-propeller	c.[409C > T]	p.[Arg137Trp]	c.[409C > T]	p. [Arg137Trp]
2	30 31	β-propeller	c.[680 A > C]	p.[His227Pro]	c.[1749G > A]	p.[Trp583*]
3	16 17	β-propeller	c.[1042C > T]	p.[Pro348Ser]	c.[2203-3C > G]	p.[?]
4	70 71	β-propeller/ Sec39	c.[1018G > C]	p.[Gly340Arg]	c.[2674G > T]	p.[Val892Phe]
5	12 64	β-propeller-Sec39	c.[1550G > A]	p.[Arg517His]	c.[6805G > T]	p.[Glu2269*]
6	55 56	Sec39	c.[2330C > A]	p.[Pro777His]	c.[2330C > A]	p.[Pro777His]
7	18 133 134	Sec39	c.[1187G > A]	p.[Trp396*]	c.[2330C > A]	p.[Pro777His]
8	58 59	Sec39	c.[3386C > T]	p.[Ser1129Phe]	c.[3386C > T]	p.[Ser1129Phe]
9	101 102	Sec39	c.[2563_c.2577 + 5del]	p.[His855_Gln859del]	c.[3596G > A]	p.[Cys1199Tyr]
10	60 88	Sec39	c.[1342-6A > G]	p.[?]	c.[3534C > A]	p.[Ser1178Arg]
11	42 43	C-terminal	c.[5741G > A]	p.[Arg1914His]	c.[6572 + 1delG]	p.[?]
12	47 48	C-terminal	c.[6237-3C > G]	p.[?]	c.[6237-3C > G]	p.[?]
13	113 114	C-terminal	c.[5741G > A]	p.[Arg1914His]	c.[del exon 35–47]	p.[?]
14	85 120	C-terminal	c.[5741G > A]	p.[Arg1914His]	c.[686dupT]	p.[Ser230Glnfs*4]

PID patient identification number.

Table 2
NBAS variants (NM_015909.3; NP_056993.2) of all individuals included in this study sharing one missense variant with a loss-of-function variant in trans.

Genotype	PID	Region/ domain of NBAS affected	Allele 1 nucleotide change	Allele 1 protein change	Allele 2 nucleotide change	Allele 2 protein change
M-1	22	β-propeller	c.[284C > T]	p.[Ala95Val]	c.[850 A > T]	p.[Lys284*]
	46				c.[2802G > A]	p.[Trp934*]
M-2	7	β-propeller	c.[1241C > T]	p.[Ser414Phe]	c.[2950delA]	p.[Ile984Leufs*8]
	8				c.[del exon 48]	p.[del exon 48]
M-3	9	Sec39	c.[2951 T > G]	p.[Ile984Ser]	c.[2827G > T]	p.[Glu943*]
	15				c.[1533_1545del]	p.[Ile512Thrfs*4]
M-4	5	Sec39	c.[3164 T > C]	p.[Leu1055Pro]	c.[3010C > T]	p.[Arg1004*]
	20				c.[686dup]	p.[Ser230Glnfs*4]
	69				c.[del exon 42–44]	p.[?]
M-5	21	Sec39	c.[3363 A > G]	p.[Ile1121Met]	c.[173-2 A > G]	p.[?]
	84				c.[513 + 2 T > C]	p.[?]
M-6	35	Sec39	c.[3596G > A]	p.[Cys1199Tyr]	c.[6611_6612insCA]	p.[Met2204Ilefs*3]
	36				c.[586C > T]	p.[Gln196*]
	44				c.[209 + 1G > A]	p.[?]
	90				c.[6751_6754delCT]	p.[L2251Cfs * 5]
	97				c.[426C > G]	p.[Tyr142Ter]
	99				c.[648-1G > A]	p.[?]
	101[a]				c.[2563_c.2577 + 5del]	p.[His855_Gln859del]
	102[a]				c.[2563_c.2577 + 5del]	p.[His855_Gln859del]
	103				c.[3284G > A]	p.[Trp1095Ter]
	121				c.[del exon 9]	p.[216–248 del]
M-7	27	C-terminal	c.[5741C > T]	p.[Arg1914His]	c.[3010C > T]	p.[Arg1004*]
	28				c.[2032C > T]	p.[Gln678*]
	29				c.[2827G > T]	p.[Glu943*]
	42[a]				c.[6572 + 1delG]	p.[?]
	43[a]				c.[6572 + 1delG]	p.[?]
	45				c.[6496-6497insA]	p.[Ser2166Phefs* 2]
	65				c.[405G > A]	p.[Trp135*]
	67				c.[6565-6566insT]	p.[Glu2189Valfs*7]
	85[a]				c.[686dupT]	p.[Ser230Glnfs*4]
	96				c.[6433-2 A > G]	p.[Ile2199_Asn2202delins16] p.[Ile2199Tyrfs*17]
	113[a]				c.[ex. 35–47 del.]	p.[?]
	114[a]				c.[ex. 35–47 del.]	p.[?]
	115				c.[1628_1629insA]	p.[Ser544fs]
	120[a]				c.[686dupT]	p.[Ser230Glnfs*4]
	123				c.[17C > A]	P.(Ser6*)
M-8	77	C-terminal	c.[5752 A > C]	p.[Thr1918Pro]	c.[500_501delTT]	p.[Phe167Cysfs*7]
	108				c.[1177_1182delinsAGATAGA]	p.[Val393ArgfsTer2]

PID patient identification number.
[a] Also included in Table 1, sharing their genotype with another individual.

NK-cell count, limiting validity of the data (Fig. 2 and Supplementary Table S3).

3.3. Genotype-phenotype correlation in individuals sharing one missense variant with a loss-of-function variant in trans

To analyze genotype-phenotype correlations in individuals sharing one missense variant with different loss-of-function variants in trans, concordance and discordance rates of clinical characteristics were analysed. For all patients with shared missense variants, 77.3% of clinical characteristics were concordant (while 22.7% were discordant). Concordance of clinical characteristics was highest in the ILFS2 subgroup (86.0%); while for the SOPH subgroup concordance rate was 65.5% and for the combined subgroup 74.2% (Fig. 3).

Clinical characteristics concordant throughout all genotypes (100%) were the following: ALF, reduced bone mineral density, delayed closure of fontanels and high-pitched voice. High concordance rates (80 - <90%) were found for short stature, abnormality of the vertebral column, abnormality of the integument, decreased circulating IgG level, muscular hypotonia and diabetes mellitus. Concordance rates for SGA, neurodevelopment delay, optic atrophy and PHA were 60 - <80%. The clinical characteristics cELT, reduced NK-cell count and facial dysmorphism had low concordance rates (< 60%); again, validity of data on NK-cell count is limited due to lack of data. For a detailed overview of concordance rates for individual clinical characteristics see Fig. 4, detailed clinical data for each individual are shown in Supplementary Table S4.

Next, phenotypes of individuals with a homozygous missense variant were compared to phenotypes of individuals sharing this missense variant with a loss-of-function variant in trans. Eleven individuals with four different missense variants (all affecting Sec39) were included for this analysis. Phenotypic concordance was 88.2%. Five of the eleven individuals are also included in the analysis of shared genotypes (H-1 respectively genotypes 6 and 7). For details, see Supplementary Tables S5-S8 (genotypes H-1 – H-4).

3.4. Impact of emergency management protocol and genetic diagnosis on course of ALF

To assess the impact of emergency management protocol and genetic diagnosis on frequency and severity of ALF episodes, we studied the course of ALF in affected siblings: Seven individuals from the combined subgroup, eleven individuals from the ILFS2 subgroup and four individuals that could not be assigned to either subgroup (genotypes 1–10). Hence, 22 individuals were included in this analysis. An emergency management protocol was implemented in 19 individuals and included different combinations of early antipyretic therapy ($n = 18$), intravenous glucose ($n = 18$), intravenous lipids ($n = 10$), intravenous carnitine ($n = 12$), *N*-acetylcysteine ($n = 1$) and arginine ($n = 1$). In eleven patients, the diagnosis was not known at implementation. Seven

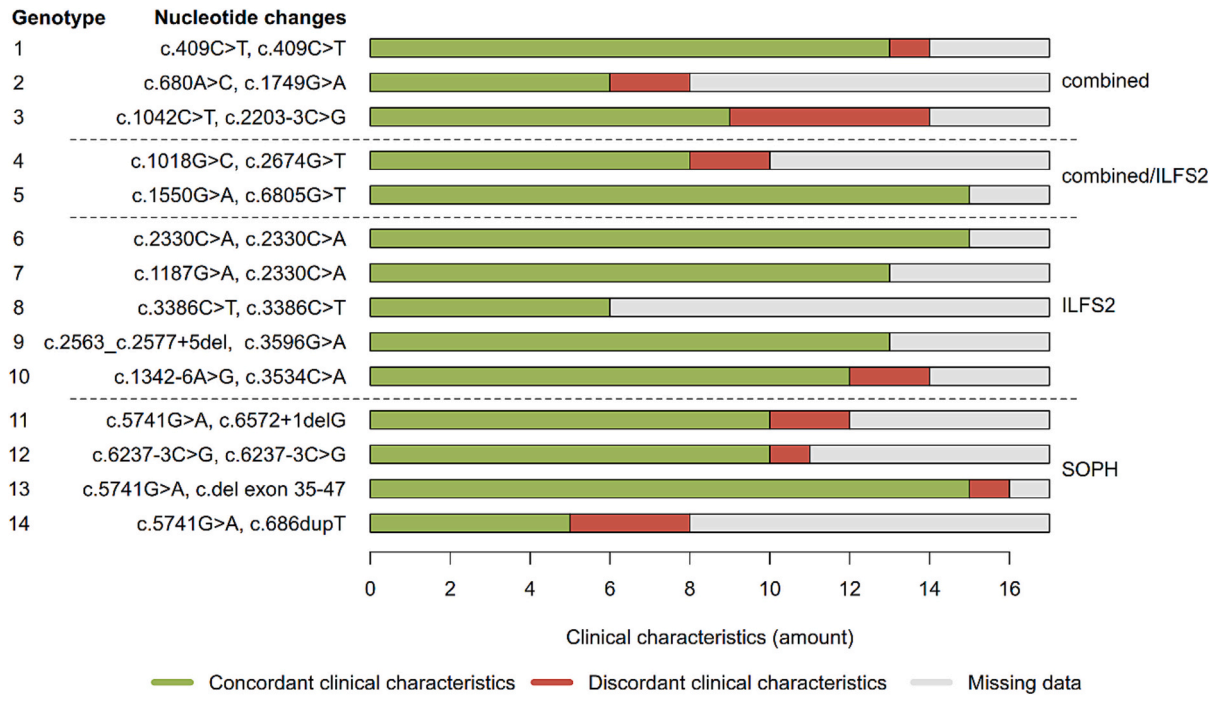

Fig. 1. Phenotypic concordance of individuals sharing the same *NBAS* genotype.
Within each of the 14 genotypes examined, concordance for the following 17 clinical characteristics was tested: *Acute liver failure, chronic elevated liver transaminases, small for gestational age, short stature (last documented value), facial dysmorphism, abnormality of the integument, abnormality of the vertebral column, reduced bone mineral density, delayed closure of fontanels, muscular hypotonia, high pitched voice, neurodevelopment delay, optic atrophy, diabetes mellitus, reduced NK-cell count, decreased circulating IgG level and Pelger-Huët anomaly*. Concordance for the characteristic is shown in green, discordance in red. Missing data are shown in white. *NBAS* variants (NM_015909.3) and allocation to one of the three subgroups combined, infantile liver failure syndrome type 2 (ILFS2) and SOPH-syndrome (short stature, optic atrophy, Pelger-Huët anomaly) are shown for all genotypes; genotypes 4 and 5 cannot be assigned to one of the subgroups combined or ILFS2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

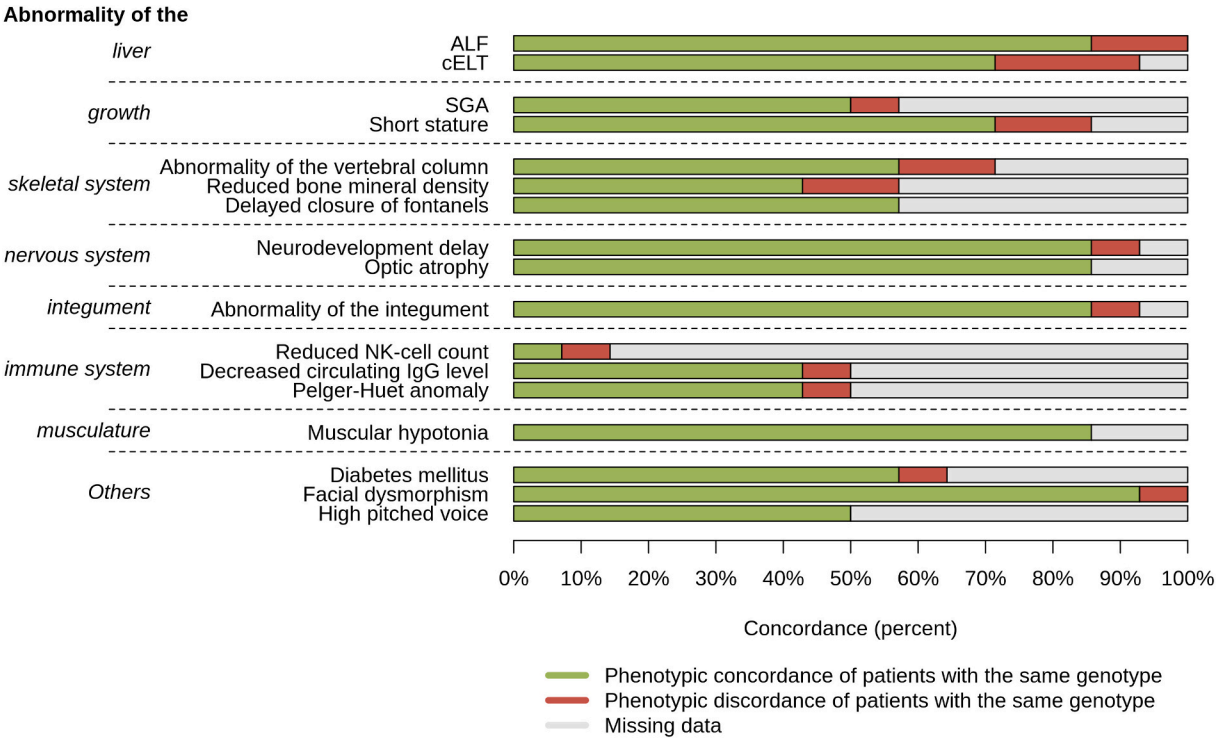

Fig. 2. Concordance of clinical characteristics among individuals sharing the same *NBAS* genotype.
Concordance for 17 clinical characteristics was tested within 14 genotypes. Concordance for the characteristic is shown in green, discordance in red. Missing data are shown in white. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
ALF acute liver failure, cELT continuously elevated liver transaminases, SGA small for gestational age, NK-cell natural killer cell, IgG immunoglobulin G.

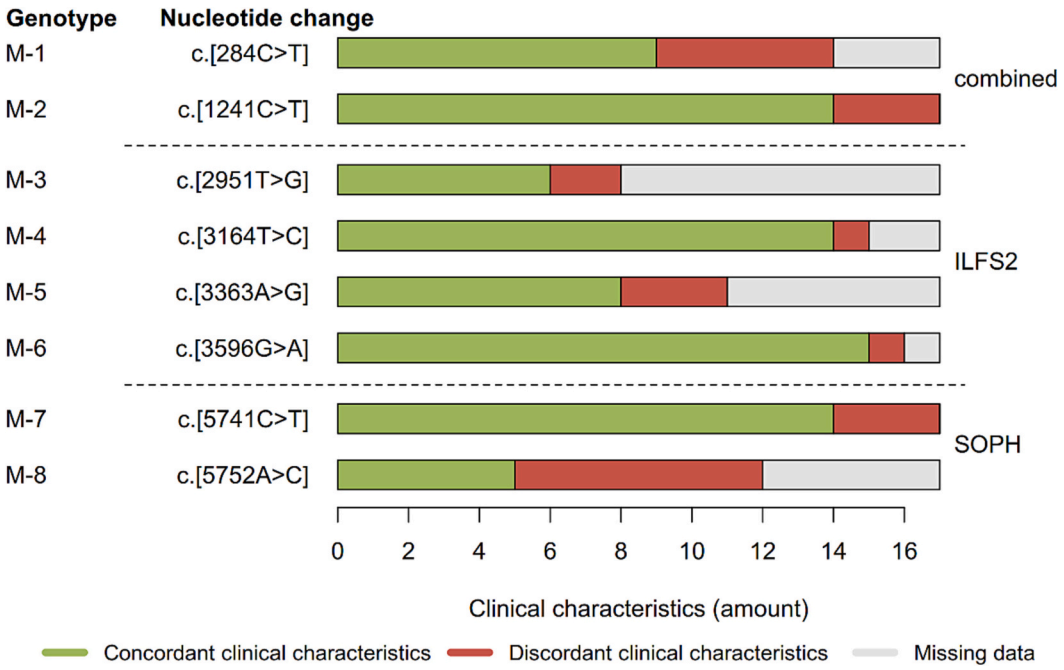

Fig. 3. Phenotypic concordance of individuals sharing one *NBAS* missense variant.
Within each of the 8 shared missense variants examined, concordance for the following 17 clinical characteristics was tested: *Acute liver failure, chronic elevated liver transaminases, small for gestational age, short stature (last documented value), facial dysmorphism, abnormality of the integument, abnormality of the vertebral column, reduced bone mineral density, delayed closure of fontanels, muscular hypotonia, high pitched voice, neurodevelopment delay, optic atrophy, diabetes mellitus, reduced NK-cell count, decreased circulating IgG level and Pelger-Huët anomaly*. Concordance for the characteristic is shown in green, discordance in red. Missing data are shown in white. *NBAS* missense variants (NM_015909.3) and affiliation to one of the three subgroups combined, infantile liver failure syndrome type 2 (ILFS2) and SOPH-syndrome (short stature, optic atrophy, Pelger-Huët anomaly) are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

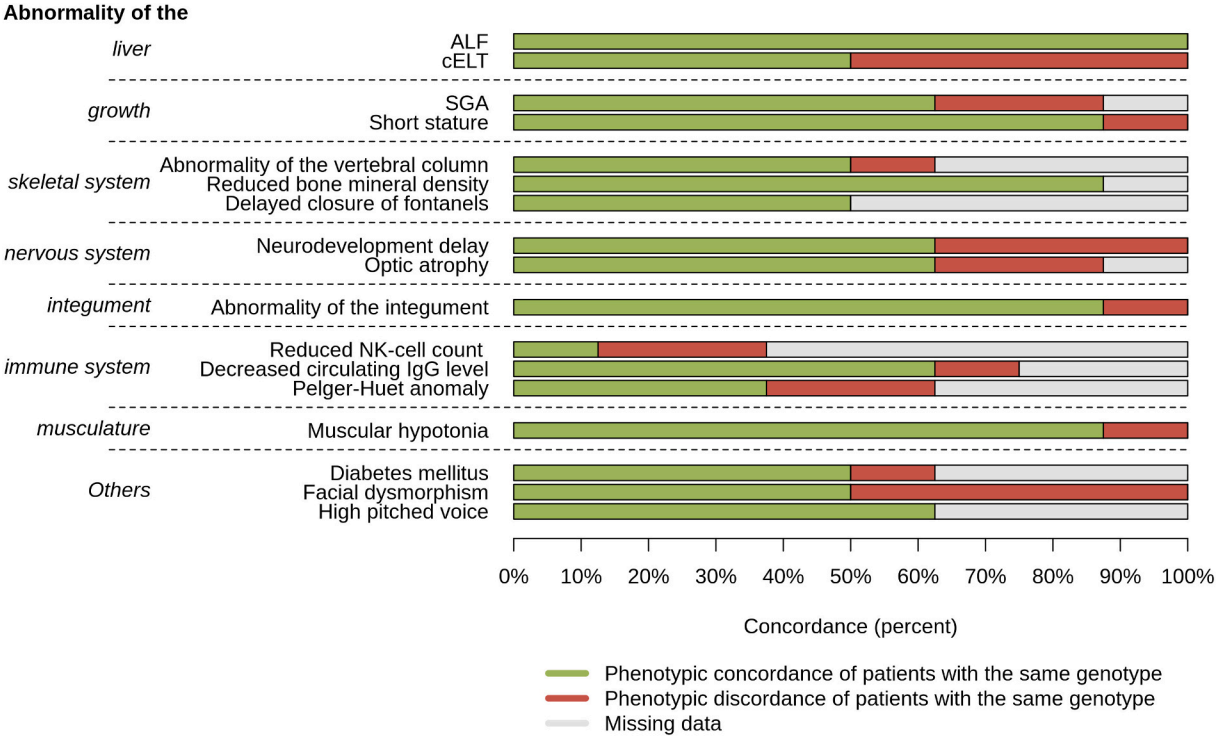

Fig. 4. Concordance of clinical characteristics among individuals sharing one *NBAS* missense variant.
Concordance for 17 clinical characteristics was tested within eight shared missense variants. Concordance for the characteristic is shown in green, discordance in red. Missing data are shown in white. ALF acute liver failure, cELT continuously elevated liver transaminases, SGA small for gestational age, NK-cell natural killer cell, IgG immunoglobulin G. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of 16 patients did not have further ALF episodes after implementation of an emergency management protocol (Table 3).

We then looked at the possible influence of the awareness of the genetic diagnosis on the occurrence of ALF. Median age at genetic diagnosis was 3.4 years (range 0.3–15.5 years) and seven individuals were diagnosed post-mortem. Nine out of 15 individuals had no further episode of ALF after the genetic diagnosis was known (Table 3). Overall, ALF still occurred despite a known genetic diagnosis and implementation of an emergency management protocol.

Five individuals were diagnosed pre-symptomatically due to family-history triggered investigation and had an emergency management protocol implemented subsequently (NBAS 64, 71, 88, 133 and 134); one of them had no episodes of ALF and four had episodes of ALF, one individual with a lethal course during the first ALF episode (NBAS64, genotype 5) (Table 3). Hence, in our cohort, even pre-symptomatically diagnosed individuals experienced ALF.

To analyze the impact of genetic diagnosis, maximum ALAT, ASAT and INR during each individual's most severe ALF episode were compared between the first (oldest) and following siblings. Overall, maximum ASAT and ALAT during ALF did not differ between the first and following siblings, values trended higher in the 2nd and 3rd sibling compared to the first sibling (ASAT: $t(10) = -0.39$; $p = 0.35$; $d = -0.19$; ALAT: $t(11) = -0.83$; $p = 0.21$; $d = -0.27$). INR as a biomarker of liver function trended lower in the 2nd and 3rd sibling with a medium effect power (INR: $t(8) = 1.20$; $p = 0.13$; $g = 0.61$). When only including families, in which the genetic diagnosis was known and an emergency management protocol was implemented prior to a first episode of ALF in the following sibling, ASAT and ALAT again did not differ (ASAT: $t(5) = -0.04$; $P = 0.48$; $d = -0.04$; ALAT: $t(5) = -0.50$; $p = 0.32$; $d = -0.38$). INR, however, was lower in the 2nd and 3rd siblings with a high effect power: $t(5) = 1.85$; $p = 0.07$; $d = 1.29$ (Fig. 5). For comparison of laboratory results between the single individuals see Supplementary Fig. S1.

Next, we investigated whether mortality was affected by pre-symptomatic genetic diagnosis and emergency management protocol. Fourteen individuals (64%) were alive with native liver survival at the

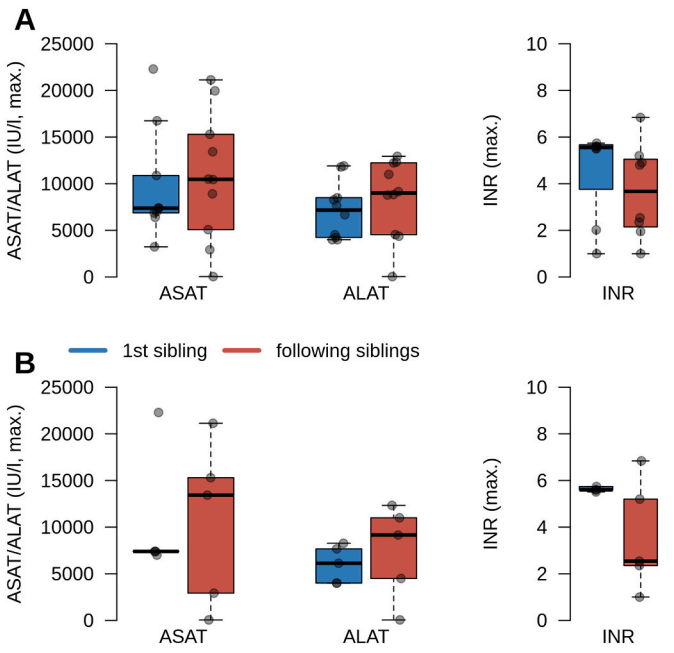

Fig. 5. Maximum ASAT, ALAT and INR during each individual's most severe episode of acute liver failure (ALF) compared between first sibling and following siblings.
Boxplots with maximum values of ASAT, ALAT and INR in relation to number in sibling sequence in (A) all siblings and (B) all siblings, where the following sibling(s) was/were diagnosed pre-symptomatically and had an emergency management protocol implemented (genotypes 4,5,7 and 10), individual data points for each individual are shown.
ASAT aspartate aminotransferase, ALAT alanine aminotransferase, INR international normalized ratio.

Table 3
ALF episodes of all individuals with at least one affected sibling, including numbers of ALF after genetic diagnosis and after implementation of an emergency management protocol.

Genotype	PID	YOB	Age at genetic diagnosis (ys)	Age at last visit (ys)	Alive, if N age at death (ys)	Use of an emergency management protocol [a], if Y age at implementation (ys)	Number of ALF episodes in total/ after the implementation of an emergency management protocol	Number of ALF episodes after genetic diagnosis
1	24	n.a.	Post-mortem	1.5	N, 1.5	Y, 0.8	n.a.* / 4–5	–
	25	n.a.	Post-mortem	3.7	N, 3.7	Y, 0.3	n.a.* / >7–8	–
	26	n.a.	Post-mortem	2	N, 2	Y, n.a.	n.a.* / n.a.	–
2	30	2012	Post-mortem	3.9	N, 3.9	N	0 / -	–
	31	2006	10	14	Y	Y, n.a.	2 / n.a.	0
3	16	2004	9	17	Y	Y, 1	4 / 4	0
	17	2002	11	19	Y	Y, 6.7	1 / 0	0
4	70	n.a.	4	7	Y	Y, 4	2 / 0	0
	71	n.a.	2	5	Y	Y, 2	0 / 0	0
5	12	2013	Post-mortem	3.4	N, 3.4	N	2 / -	–
	64	2018	0.3	1.3	N, 1.3	Y, 0.3	1 / 1	1
6	55	2015	2.8	3.4	Y	Y, n.a.	1 / n.a.	0
	56	2009	8.7	8.9	Y	Y, n.a.	1 / 0	0
7	18	2006	9.7	15	Y	Y, 8	1 / 0	0
	133	2017	2.4	4	Y	Y, 0	3 / 3	1
	134	2019	1.6	2.7	Y	Y, 0	1 / 1	1
8	58	2002	15.5	16	Y	Y, n.a.	1 / 0	0
	59	2008	Post-mortem	2.3	N, 2.3	N	2 / -	–
9	101	2015	3.4	6.2	Y	Y, 4	2 / 0	1
	102	2017	Post-mortem	1.3	N, 1.3	Y, n.a.	1 / 1	–
10	60	2017	0.8	4.3	Y	Y, 0.8	2 / 1	1
	88	2019	0.3	2.7	Y	Y, 0.3	1 / 1	1

n.a.*: at least one episode of ALF, the number of ALF episodes is unknown.
PID patient identification number, YOB year of birth, YS age in years, N no, Y yes, ALF acute liver failure, n.a. not available.
[a] emergency management protocols differed between medical centres, especially before the genetic diagnosis was known. They included a varying combination of early antipyretic therapy, iv glucose, iv lipids, and iv carnitine in most individuals.

time of data retrieval for this study, whereas eight individuals had died. None of these individuals received liver transplantation. Median age at death was 2.2 years (range 1.3 to 3.9 years). Seven individuals died due to ALF, whereas one died due to pneumonia and elevated liver transaminases without fulfilling the criteria for ALF (NBAS30). One individual died despite implemented emergency management protocol and a pre-symptomatic confirmed genetic diagnosis (one out of five, 20%) compared to six out of 17 (35.3%) individuals without pre-symptomatic genetic diagnosis and emergency management protocol; however, this difference is not statistically significant (Boschloo's test, $p = 0.61$).

4. Discussion

We present a retrospective, multicentre study on genotype-phenotype correlations in 60 individuals with NBAS-associated disease. Among individuals sharing the same *NBAS* genotype ($n = 30$), we found a high concordance of clinical symptoms of nearly 90%, whereas concordance was lower (nearly 80%) in individuals sharing one missense variant with a loss-of-function variant in trans ($n = 38$). Overall, genotype-phenotype correlation was strongest for individuals belonging to the ILFS2 subgroup.

4.1. Genotype-phenotype correlation in individuals sharing the same genotype

While some clinical characteristics were concordant in all individuals sharing both *NBAS* variants (namely delayed closure of fontanels, optic atrophy, muscular hypotonia and high-pitched voice), and thus seem to not be influenced by other genetic or environmental factors, other characteristics as cELT showed low rates of concordance among individuals with the same *NBAS* variants. Incomplete penetrance or genetic factors other than *NBAS* variants are possible explanations for discordance of clinical symptoms, but also epigenetic or environmental factors including comorbidities and sociocultural factors (e.g. race and ethnicity, socioeconomic and demographic factors), medical therapies and symptoms that evolve with age must be considered. This had already been proposed for the immunologic phenotype including decreased IgG and reduced NK-cell count [14], which indeed is present at a higher rate with increasing age in this cohort. Similarly, diabetes mellitus seems to be developing at an older age. Conversely, some clinical characteristics appear to be becoming less evident with age or even resolve over time (e.g., cELT). Physicians' different perception of subjective clinical characteristics such as facial dysmorphism and abnormality of the integument might have a further impact on discordance. Mildly present clinical characteristics might have been overlooked or simply not been tested for (e.g., reduced bone mineral density), especially if investigations were done before NBAS-associated disease was known as disease entity.

Only one study [3] has investigated multiple individuals sharing the same *NBAS* variant, examining 33 individuals with the homozygous founder variant c.5741G > A (p.Arg1914His), affecting the C-terminal region. Those individuals were not included in our study for lack of individualized data. However, concordance for short stature, abnormality of the integument, optic nerve atrophy, PHA, facial dysmorphism, diabetes mellitus, high pitched voice and muscular hypotonia were similar to the findings from our study, whereas SGA was reported in approximately 50% of individuals [3]. No information was given for some other typical clinical characteristics including ALF, cELT and reduced bone mineral density.

The strong genotype-phenotype correlation in specific genotypes supports prediction of the clinical course of a newly diagnosed individual and guides diagnostics and monitoring, when patients with the same genotype are already known or published; this holds true especially in families with an affected sibling.

4.2. Genotype-phenotype correlation in individuals sharing a missense variant with a loss-of-function variant in trans

Among individuals sharing one missense variant with a loss-of-function variant in trans, concordance of clinical symptoms was still high but at a decreased level with nearly 80%. This indicates that loss-of-function *NBAS* variants have some impact on the phenotype, contradicting our previous observation that the missense variant or in-frame deletion determines the phenotype regardless of the loss-of-function variant in trans [1]. However, the fact that siblings share considerably more genetic and environmental factors than unrelated children possibly contributes to the higher rate of consistency of symptoms in individuals sharing the same genotype compared to those sharing one missense variant (with more unrelated individuals included). The role of splice variants remains to be determined, as they might lead to alternative spliced RNA products that escape nonsense mediated decay [1]; however, we did not observe a difference in dependency of the type of loss-of-function variant in trans (premature termination of translation, splice site effect or exon deletion). In conclusion, in individuals sharing one missense variant with different loss-of-function variants in trans, phenotypic prediction is more challenging, as the loss-of-function variant impacts clinical phenotype.

4.3. Impact of emergency management protocol on severity of ALF episodes

Despite a known genetic diagnosis and implementation of an emergency management protocol, individuals continued having ALF episodes. Hence knowledge of genetic diagnosis and emergency management protocol do not prevent ALF. Probability of ALF cannot be calculated in more detail, as likelihood of ALF decreases with increasing age; in addition, monitoring and thereby detection rate of ALF can be influenced by knowledge of the disease aetiology. Number of ALF episodes did not differ between younger and older siblings, nor did maximum ASAT or ALAT levels. However, there is a difference in maximum INR between older siblings and younger siblings with a pre-symptomatic emergency protocol, which although not statistically significant, has a high effect power, indicating that ALF was less severe in younger siblings. Pre-symptomatic genetic diagnosis and emergency management protocol were not significantly associated with reduced mortality in this cohort. However, the only individual (NBAS64) who died despite pre-symptomatically known genetic diagnosis and emergency management protocol came to medical attention late in the course of ALF, demonstrating that the course of liver crises is also determined by access to medical care. Several reports of families with fatal outcome in the first siblings and survival in the following support an impact of known genetic diagnosis and possibly emergency management protocol on reduction of mortality (e.g. [1,4,25,33–35]). These cases have not been included in the current study, as the diagnosis of the deceased children was not ascertained genetically. Overall, our data indicate a possibly ameliorated course of ALF in individuals with ILFS2 and with a combined phenotype with pre-symptomatic genetic diagnosis and an implemented emergency management protocol. We were not able to proof that prophylactic antipyretic treatment prevents ALF in ILFS2, as the occurrence of ALF itself was not influenced by knowledge of genetic diagnosis and emergency management protocol. The effect of a defined emergency protocol remains to be evaluated.

4.4. Strengths and limitations

This is the first study systematically examining genotype-phenotype correlations in NBAS-associated disease in individuals sharing the same genotype. It includes updated clinical information of most individuals, assessed via standardized CRFs, also examining effects of pre-symptomatic diagnosis and implementation of an emergency management protocol on ALF. However, for each genotype, there are only few

individuals (mostly two) compared, most of whom being siblings. Thus, genotype-phenotype corelations in this study are influenced by the various genetic and environmental common features shared by siblings. For some clinical characteristics (especially NK-cell count) data availability was poor, limiting our results. Furthermore, we did not include severity of clinical characteristics in our analysis of concordance. Impact of liver transplantation on the phenotype and thus a possible confounding effect was not examined; however, only one out of 60 individuals was transplanted. Small numbers did not allow statistical testing of an impact of emergency treatment, which in addition was heterogenous.

5. Conclusion

Overall, we demonstrate a strong genotype-phenotype correlation in individuals sharing the same genotype, especially in individuals with ILFS2. This will improve monitoring of affected patients and counselling families. Genotype-phenotype correlation is less pronounced in individuals sharing one missense variant with different loss-of-function variants in trans, pointing at an impact of the loss-of-function variant in trans. Our results indicate an ameliorated course of ALF episodes in patients with pre-symptomatic diagnosis and implementation of an emergency management protocol, highlighting the importance of early diagnosis and disease specific management in NBAS-associated disease.

CRediT authorship contribution statement

Nicole Hammann: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Methodology. **Dominic Lenz:** Conceptualization, Data curation, Writing – review & editing, Investigation, Methodology, Supervision, Funding acquisition. **Ivo Baric:** Data curation, Writing – review & editing. **Ellen Crushell:** Data curation, Writing – review & editing. **Carlo Dionisi Vici:** Data curation, Writing – review & editing. **Felix Distelmaier:** Data curation, Writing – review & editing. **Francois Feillet:** Data curation, Writing – review & editing. **Peter Freisinger:** Data curation, Writing – review & editing. **Maja Hempel:** Data curation, Methodology, Writing – review & editing. **Anna L. Khoreva:** Data curation, Writing – review & editing. **Martin W. Laass:** Data curation, Writing – review & editing. **Yves Lacassie:** Data curation, Writing – review & editing. **Elke Lainka:** Data curation, Writing – review & editing. **Catherine Larson-Nath:** Data curation, Writing – review & editing. **Zhongdie Li:** Data curation, Writing – review & editing. **Patryk Lipiński:** Data curation, Writing – review & editing. **Eberhard Lurz:** Data curation, Writing – review & editing. **André Mégarbané:** Data curation, Writing – review & editing. **Susana Nobre:** Data curation, Writing – review & editing. **Giorgia Olivieri:** Data curation, Writing – review & editing. **Bianca Peters:** Data curation, Methodology, Writing – review & editing. **Paolo Prontera:** Data curation, Writing – review & editing. **Lea D. Schlieben:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Christine M. Seroogy:** Data curation, Writing – review & editing. **Cristina Sobacchi:** Data curation, Writing – review & editing. **Shigeru Suzuki:** Data curation, Writing – review & editing. **Christel Tran:** Data curation, Writing – review & editing. **Jerry Vockley:** Data curation, Writing – review & editing. **Jian-She Wang:** Data curation, Writing – review & editing. **Matias Wagner:** Data curation, Writing – review & editing. **Holger Prokisch:** Data curation, Investigation, Methodology, Writing – review & editing. **Sven F. Garbade:** Formal analysis, Investigation, Methodology, Writing – review & editing, Visualization. **Stefan Kölker:** Data curation, Writing – review & editing. **Georg F. Hoffmann:** Writing – review & editing, Data curation. **Christian Staufner:** Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing, Funding acquisition.

Declaration of competing interest

None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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