ARTICLE

Check for updates

Comprehensive analysis of *CNOT3*-related neurodevelopmental disorders: phenotypic and genotypic characterization

Camille Engel[1,2✉], Michaela Rendek[1], Jessica Assoumani[1], Emanuela Argilli[3], Francesca Ariani[4,5,6], Anne-Laude Avice-Denizet[1], Emilia K. Bijlsmaa[7], Pierre Blanc[8], Lucia Pia Bruno[4,5,9], Bert Callewaert[10], Valeria Capra[11], Michele Carullo[12], Bertrand Chesneau[13], Sandra Coppens[14], Cynthia Curry[15], Breanne Dale[16], Eric Dahlen[17], Andrée Delahaye-Duriez[18,19,20], Anne-Sophie Denommé-Pichon[2,21], Bénédicte Demeer[22], Lenka Dvořáková[23], Jan Fischer[24], David Geneviève[25], Thea Giacomini[26,27], Mette M. Handrup[28], Delphine Heron[29], Irina Hüning[30], Michelle Iacomino[31], Bertrand Isidor[32], Boris Keren[29], Stanislav Kmoch[33], David A. Koolen[34], Andrea Kübler[24], Jana Laštůvková[35], Carolyn Le[3], Jonathan Levy[36], Caterina Lo Rizzo[6], Silvia Maitz[37], Sandrine Marlin[38,39], Cyril Mignot[29], Ghayda Mirzaa[40,41,42], Inga Nagel[43], Sebastian Neuens[44], Lenka Nosková[33], Emily Pao[41], Anna Pecková[35], Julie Plaisancie[13], Joseph Porrmann[24], Flavia Privitera[45], André Reis[46,47], Alessandra Renieri[4,5], Marlène Rio[48], Alyssa Rippert[49], Lukáš Ryba[23], Marcello Scala[26,31], Jolanda H. Schieving[50], Elliott H. Sherr[3], Andrew Shuen[51], Richard Sidlow[52], Thomas Smol[53], Julie Soblet[44,54], Pasquale Striano[26,55], Mohnish Suri[56], Hannes Syryn[57,58], Frédéric Tran Mau-Them[2,21], Andre M. Travessa[59,60], Julien Van Gils[61], Georgia Vasileiou[46,47], Jolijn J. A. Verseput[34], Catheline Vilain[44], Catherine Vincent-Delorme[62], Emílie Vyhnálková[23], Emma L. Wakeling[63], Pia Zacher[64], Federico Zara[26,31], Paul Kuentz[2,17] and Juliette Piard[1,2✉]

The CCR4-NOT complex, crucial in gene expression regulation, includes CNOT3, a subunit linked to neurodevelopmental disorders when mutated. This study investigates 51 patients from 42 families with heterozygous *CNOT3* variants, aiming to expand the understanding of *CNOT3*-related neurodevelopmental disorders and explore genotype-phenotype correlations. Patients originated from various countries, reflecting the disorder's global significance. All patients exhibited developmental delays, particularly in the language area. Intellectual disability was found in 87% of patients and was typically mild to moderate. Behavioral issues, including autism spectrum disorders and attention deficits, were common, affecting over half of the patients. Dysmorphic features were highlighted and may help establishing the diagnosis. Epilepsy was uncommon (10%). Twenty-eight novel variants were identified, including missense, nonsense, frameshift, intronic variations and a deletion of 12 exons. Missense variants clustered at the N- and C-terminal regions of the protein, indicating critical functional roles. No clear genotype-phenotype correlation was observed, suggesting that all identified variants resulted in a loss-of-function effect. Finally, this work delineates the clinical and molecular spectrum of *CNOT3*-related disorders thanks to an in-depth characterization of a large cohort. Further research will be necessary to understand the functional consequences of the variants and enhance patient long-term outcomes.

European Journal of Human Genetics (2025) 33:989–996; https://doi.org/10.1038/s41431-025-01884-z

INTRODUCTION

The CCR4-NOT complex, a pivotal regulator of gene expression, is involved in various cellular processes, including cell cycle regulation, activation and inhibition of transcript initiation, control of transcription elongation, mRNA export, nuclear control, and DNA damage repair [1]. This complex is composed of 8 subunits, including CNOT3, which binds to the ribosome. De novo heterozygous loss-of-function *CNOT3* variants were first described by Martin et al. [2] in 2018 in 16 individuals presenting with hypotonia, relatively short stature, developmental delay, behavioral disorders and intellectual disability. Two years later, Meyer et al. [3] reported two familial cases with dominantly segregating *CNOT3* variants and variable intra-familial phenotypes. So far, 31 individuals from 25 families have been described with *CNOT3* heterozygous variants [2–8].

Here, we report a large cohort of 51 additional individuals from 42 families. We aim to further delineate the spectrum of *CNOT3*-related neurodevelopmental disorders and discuss genotype-phenotype correlations.

SUBJECTS AND METHODS

Individuals

Individuals with heterozygous *CNOT3* variants originated from Belgium, Canada, Czech Republic, Denmark, France, Germany, Italy, the Netherlands, Portugal, United Kingdom and United States. Clinicians and biologists from different centers were contacted through GeneMatcher [9], DECIPHER [10], the French ANddi-RAres healthcare network and the European Reference Network (ITHACA). Clinical and molecular data were provided by the referring clinicians in a detailed table (Supplementary Data S1) and a summarized free text (Supplementary Data S4). Written informed consent

A full list of author affiliations appears at the end of the paper.

Received: 23 June 2024 Revised: 8 February 2025 Accepted: 20 May 2025
Published online: 25 June 2025

was obtained for genetic testing and the use of photographs (if applicable). In addition, published clinical articles [2–7] were reviewed on PubMed up to the beginning of January 2024 using the keyword "CNOT3" (Supplementary data S2).

P3 and P43, previously reported in the literature by Martin et al. [2] (respectively as individuals P4 and P13) were included in our cohort with updated and extensive clinical data.

METHODS

CNOT3 variants were identified using exome sequencing in 38 individuals, by targeted gene panels for 5 individuals, and through whole genome sequencing for 2. Segregation analyses were performed by targeted Sanger sequencing and confirmed the diagnosis in 6 family members.

CNOT3 variants are given according to the mRNA reference number NM_014516.4. The bioinformatics data were obtained from Mobidetails interface [11] (CADD (v1.6) [12], Polyphen-2 [13], SIFT scores [14], and ClinVar (v20230819) [15], LOVD [16], dbSNP (v.156) [17] and gnomAD (v4.1.0) [18] databases), UCSC database [19] (GERP score [20]), Grantham matrix [21] and PROVEAN score [22]. The effect on splicing was analyzed using SPIP (v2.1) [23], RDDC RNA Splicer [24], splice AI score [25] and the UCSC database (Supplementary data S3). The graphical representation of the protein variants was created using IBS 1.0.3 software [26].

Only likely pathogenic (LP) or pathogenic (P) variants according to the ACMG guidelines were retained for this study [27]. The Franklin interface [28] was used to classify the variants.

Individuals with variants of unknown significance according to the ACMG criteria were not included in this study. To ensure consistency, we applied the same criteria to the individuals previously reported in the literature, and therefore excluded individuals with c.355 A > G; p.(Lys119-Glu) and c.2090 G > A; p.(Arg697Gln) variants reported by Martin et al., c.2017_2019del; p.(Phe673del) reported by Lee et al. [8]. as well as c.910 G > A; p.(Gly304Ser) and c.1318 G > A; p.(Leu522Ile) variants reported by Pinard et al. [4].

Variants with supportive splicing predictions by both Splice AI (> 0.2) and Spip (>80%) were considered as possibly impacting splice variants. This corresponds to the following variants in our cohort: c.25 G > C; p.(Gly9Arg), c.26-2 A > G ; p.(?), c.93 G > A ; p.(Lys31 =), c.258 G > A; p.(Thr86 =), c.387+1 G > A; p.(?), c.1406+1 G > A; p.(?), c.1705+2 T > G ; p.(?) and c.1904+2 T > C; p.(?) and to the variant c.387+2 T > C ; p.(?) in the literature data. The RNA sequence has been studied in silico (splice AI and RDDC), and the majority of variants appear to result in a frameshift, with the exception of two variants that could potentially result in an inframe deletion. As these are only predictions, we decided to consider these splice variants separately and not as truncating or missense variants.

RESULTS

Clinical data (Table 1)

Thanks to an international collaboration, we collected in-depth clinical data from 51 new individuals with *CNOT3* P/LP variants. Families were of West and Central Europe (Belgium, Czech Republic, France, Germany, Italy, the Netherlands, Portugal, the UK), North Africa (Algeria), Middle East (Türkiye), South Asia (Thailand, Sri Lanka), Mayotte and North American descent. Consanguinity was noted in 1 family. Twenty-two individuals were female and 29 were male. The median age at last follow-up was 10 years (11 months – 36 years).

Pregnancy was uneventful for both mother and fetus in 57% of cases (28/49). One pregnancy resulted from in vitro fertilization. Four twin pregnancies were included (one with fetal resorption at 7 weeks' gestation). Gestational complications were observed in ten women, with seven developing gestational diabetes, one with gestational hypertension, another having a deep vein thrombosis, and one with a fever at 4 months of pregnancy. Ultrasound detected fetal anomalies in 34% of pregnancies (16/47), mostly growth restriction or IUGR (13/47; 28%), including three from twin pregnancies. IUGR was associated with maternal smoking in two cases, oligohydramnios in two other cases, and pre-anasarca in one pregnancy. Other fetal findings included isolated oligohydramnios, polyhydramnios with duodenal atresia, dilatation of the lateral ventricle, and a cerebral cyst, each observed in one fetus. Amniocentesis was performed in three pregnancies with cerebral or gastrointestinal ultrasound anomalies. Delivery by Cesarean section was performed in 36% of cases (15/42), half of which were urgent (9/15; 60%). Prematurity (birth before 37-weeks of gestation) was observed in 15 individuals (16/49; 33%). The neonatal period was marked by hypotonia in 43% of cases (18/42) and feeding difficulties in about half the cases (26/47; 55%).

All patients had a history of neurodevelopmental delay (47/47; 100%). Of the remaining 4 patients, early childhood informations were not available for 3, and one patient is under one year old. In most cases, developmental delay was global and more pronounced in the language area. Gross motor delay affected 88% of individuals (42/48). Ninety-eight percent of them acquired independent sitting (49/50), in 41% of cases after 10 months (12/29). Ninety-eight percent acquired the ability to walk (47/48), in 63% of cases after 18 months (28/46). The individual who was not able to walk was 18 months old at the time of the study. Difficulties with fine motor skills were reported in 33 individuals in our cohort (33/35; 94%). All individuals had language delay (44/44; 100%). Of the 7 individuals for whom we have no information on language skills, two are less than one year old and five are adults for whom childhood data were not available. Most individuals could say a few words (41/49; 84%), while two-thirds were capable of using word associations (35/48; 73%) or form sentences (32/48; 67%). Speech therapy was necessary in the majority of cases (36/41; 88%).

Intellectual disability was found in 87% of individuals (33/38). Thirteen individuals were too young to be assessed. ID was mild to moderate in the most cases (19/33; 58% and 12/33; 36% respectively). Two individuals had severe ID and remained non-verbal. Of the five individuals without a diagnosis of ID, two had borderline IQs, two others had low IQs, one of which combined with a language disorder, and the last one had a heterogeneous IQ. Of the 44 individuals who are either currently enrolled in school or previously attended school, 33 required specialized schooling (33/44; 75%) and 11 needed human or material support at a mainstream school (11/44; 25%).

Twenty-eight patients in our cohort exhibited autistic features, with 12 formally diagnosed with autism spectrum disorder (25%) and 16 displaying significant autistic traits (33%). These individuals were described as having difficulties in social interaction (18/28; 64%), resistance to change (15/28; 53%), stereotypies (15/28; 53%), abnormal affect (15/28; 53%), and restricted interests (14/28; 50%). Twenty-one individuals had an attention deficit disorder with or without hyperactivity, either formally diagnosed or not (21/38; 55%) and 27 individuals had behavioral disturbances (28/45; 62%) including intolerance to frustration (14/26; 54%), repeated tantrums (9/26; 35%), hetero-aggressivity (10/26; 38%) and oppositional behavior (7/26; 27%). Anxiety and sleep disorders were respectively reported in 8 and 10 individuals (8/37; 22% and 10/39; 26%).

In our cohort, data from 10 adult individuals (>18 years old) were collected. Nine of them had intellectual disability and attended specialized schooling. Five of them worked in sheltered places, two attempted sheltered work, but were unsuccessful, one is in job training and the last one had just turned 18. The tenth adult individual had a borderline IQ, she obtained a middle school diploma but had a much lower level of education than her relatives. She currently works as an employee. Curatorship or guardianship was required for four individuals (4/8; 50%). Five adult individuals are able to live independently, while three live in sheltered homes and the two youngest still live with their parents. Four have children, who are included in this cohort.

Five individuals in our cohort had epileptic seizures (5/51; 10%). One had a simple generalized convulsive seizure at 2 days of age, without recurrence; two had a few episodes of generalized tonic-clonic seizures and are currently seizure-free without treatment; one presented with long-term epilepsy starting at the age of 4,

Table 1. Clinical data of the 50 individuals with *CNOT3* heterozygous variants.

	Our cohort		Literature		Total	
Number of families	42		20		62	
Number of patients	51		24		75	
Pregnancy and birth						
IUGR or growth retardation	13/47	28%	2/3	67%	15/50	30%
C-section	15/42	36%	3/16	19%	18/58	31%
Prematurity	16/49	33%	3/21	14%	19/70	27%
Neonatal hypotonia	18/42	43%	2/4	50%	20/46	43%
Feeding difficulties	26/47	55%	2/5	40%	28/52	54%
Developmental delay	47/47	100%	21/21	100%	68/68	100%
Motor delay	43/49	88%	17/18	94%	60/67	90%
Language delay	44/44	100%	20/20	100%	64/64	100%
Acquisitions, schooling and work						
Intellectual disability	33/38	87%	12/12	100%	45/50	90%
Mild	19/33	58%				
Moderate	12/33	36%				
Severe	2/33	6%				
Schooling	44/50	88%	11/11	100%	55/61	90%
Need for support in mainstream schooling	11/44	25%	2/10	20%	13/54	24%
Specialized schooling	33/44	75%	8/11	73%	41/55	75%
Ability to work	6/10	60%	1/1	100%	7/11	64%
Sheltered employment	5/6	83%				
Ability to live independently	5/10	50%				
Need for curatorship/guardianship	4/8	50%				
Behavior and neuropsychiatric disorders						
Autism spectrum disorder	12/48	25%	6/8	75%	18/56	32%
Autistic features w/o formal ASD diagnosis	16/48	33%	3/5	60%	19/53	36%
Attention deficit hyperactivity disorder	21/38	55%				
Behaviouhal disturbances	28/45	62%				
Neurological or sensory conditions						
Seizures	5/51	10%	4/24	17%	9/75	12%
Hearing loss	7/51	14%	2/23	9%	9/74	12%
Strabismus	14/44	32%	10/17	59%	24/61	39%
Organ malformations						
Cardiac anomalies	4/46	9%	4/24	17%	8/70	11%
Gastrointestinal malformations	4/39	10%	2/24	8%	6/63	10%
Genital anomalies	3/42	7%	2/24	8%	5/66	8%
Physical examination						
Macrocephaly (OFC > +2 SD)	5/47	11%	0/19	0%	5/66	8%
Microcephaly (OFC < -2SD)	6/47	13%	1/19	5%	7/66	11%
Small stature (height < -2SD)	12/50	24%	10/23	43%	22/73	30%
Hypotonia	21/43	49%	9/16	56%	30/59	51%
Cerebral MRI	32/50	64%	14/23	61%	46/73	63%
Normal	10/32	31%	4/14	29%	14/46	30%
Corpus callosum anomalies	13/32	41%	5/14	36%	18/46	39%
Posterior fossa anomalies	5/32	16%	1/14	7%	6/46	13%
Ventriculomegaly / Hydrocephalus	5/32	16%	1/11	9%	6/43	14%

stabilized on bitherapy ; and the fifth individual had West syndrome in the first months of life, in a context of prematurity with intraventricular hemorrhage, and died at 6 months. An additional individual suffered a single febrile seizure at 14 months of age and another presented 2 episodes of unconfirmed seizures; they were therefore not considered to have epilepsy.

Brain magnetic resonance imaging (MRI) was performed in 64% of the cases (32/50) and was normal in one third of individuals (10/32; 31%). Corpus callosum anomalies were found in 41% of cases (13/32) (hypoplasia in 8, dysmorphism in 5 and partial or total agenesis in 3) and posterior fossa anomalies in 5 (5/32; 16%). Ventriculomegaly was reported in 4 individuals, cerebral atrophy

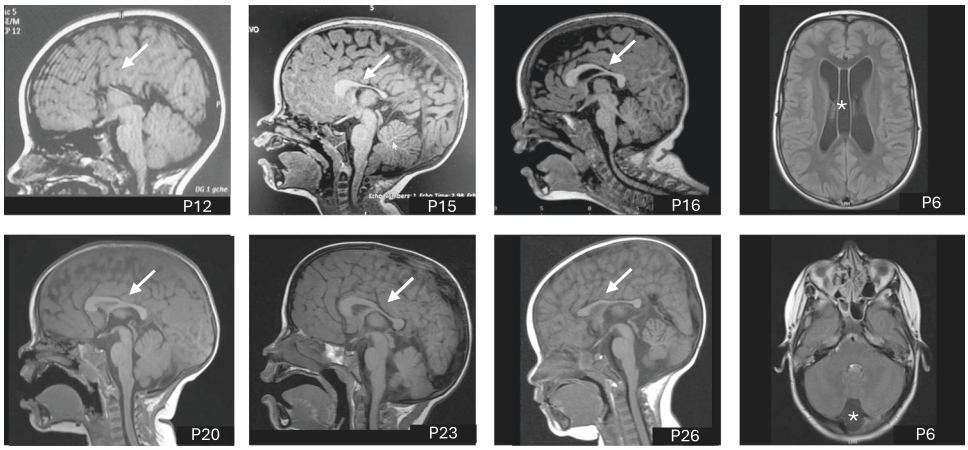

Fig. 1 Brain magnetic resonance image (MRI) of individuals with *CNOT3* variants. Note hypoplastic, absent and dysmorphic corpus callosum (white arrows), septum pellucidum cyst and retrocerebellar cysts (white asterisks).

or dilatation of pericerebral spaces in 3 and arachnoid cysts in 3 others. Two individuals have periventricular leukomalacia and intraventricular hemorrhage in the context of prematurity. Other brain MRI signs, each found in a single individual, were thin white matter, ventricular asymmetry, partial thrombosis of the sinus sagitalis, periventricular hyperintensity, thin optic nerve and small pituitary gland (Fig. 1).

Congenital birth defects included cardiac anomalies in 4 individuals (atrial septum defect in two, ventricular septum defect in one and patent foramen ovale in another), gastro-intestinal defects in 4 cases (anal atresia in a proband and his mother, esophageal stenosis in one individual and duodenal atresia in another) and genital anomalies in 3 cases (cryptorchidism in 3 male individuals associated with hypospadias in one case).

Twelve people showed a susceptibility to infections, with six suffering from upper respiratory tract infections, four from ear infections and two from urinary tract infections. Osteoarticular anomalies included brachydactyly (8/42; 19%), tapering fingers (6/42; 14%), single transverse palmar crease (6/42; 14%), flat feet (9/39; 23%), hyperlordosis (4/40; 10%) and scoliosis (3/40; 8%). Eleven individuals had umbilical or inguinal hernias (respectively 3/49; 6% and 8/42; 19%), and 6 had joint hyperlaxity (6/43; 14%).

Bilateral sensorineural hearing loss requiring hearing aids was reported in two individuals, one occurring before the age of 2 and the other during adulthood. A third individual presented with a moderate unilateral mixed hearing loss from the age of 4 and also benefited from hearing aids. A 4th individual had congenital unilateral profound deafness linked to cochleovestibular nerve agenesis. Three other individuals had conductive hearing loss, normalized by grommets in two cases.

Ophthalmological signs included strabismus in 14 individuals (14/44; 32%), refractive abnormalities in 11 (11/41; 27%), optic nerve injury in 5 (5/38; 13%) and nystagmus in 3 (3/42; 7%). Amblyopia and lacrimal duct obstruction were also reported in two individuals each.

On clinical examination, short stature (<−2 SD) was observed in 24% of patients (12/50), microcephaly (OFC < −2 SD) in 13% (6/47), and macrocephaly (>+2 SD) in 11% (5/47). Hypotonia was noted in 21 individuals (21/42; 50%) and clumsiness in 12 (12/39; 31%). The most common morphological features were a high, broad and/or prominent forehead in 72% of individuals (34/47), upslanting palpebral fissures in 48% (22/46), a thin upper lip in 48% (22/46) and variable nose anomalies (39/45; 87%), including short nose in 17 individuals, low-hanging columella in 17, broad nasal tip in 16, alae nasi hypoplasia in 14, and anteverted nostrils in 12. Other characteristics frequently observed were deep-set eyes or enophtalmia (26%; 12/46), protruding, dysplastic or low-set ears (55%; 24/44) and a smooth or short philtrum (18/43; 42%) (Fig. 2).

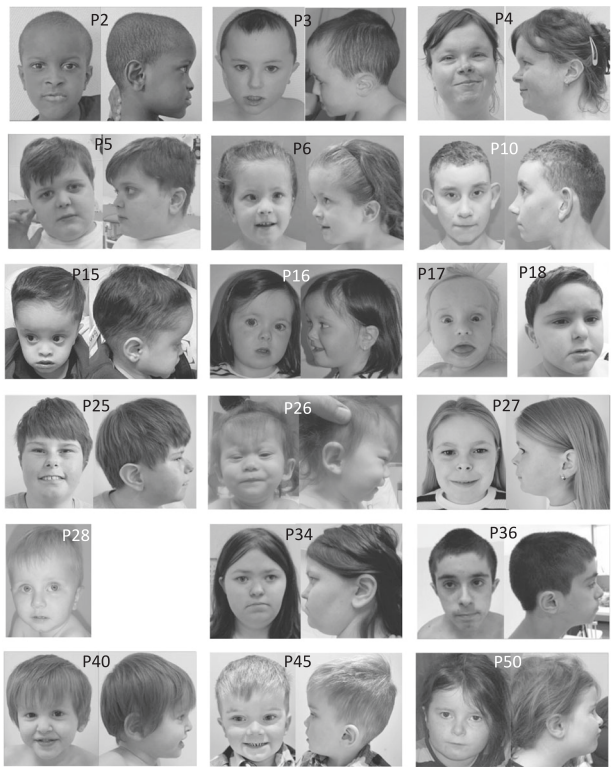

Fig. 2 Photographs of individuals with *CNOT3*-related NDD. Note recurrent signs as high, wide and prominent forehead, upslanting palpebral fissures, dysplastic and/or posteriorly rotated ears, low hanging columella, anteverted nares, smooth philtrum and thin upper lip.

Molecular findings

In addition to the 20 pathogenic/likely pathogenic variants previously published, we identified a deletion of 12 exons and 27 new SNVs in our cohort, including 9 frameshifts, 6 missense, 5 intronic variants, 4 nonsense, 2 synonymous variants, and 1 in-frame deletion (Fig. 3).

Three *CNOT3* variants were recurrent in our cohort: c.732dup; p.(Ser245fs) was found in 5 families, c.563 G > A; p.(Arg188His) and c.1979_1980del; p.(Val660GlyfsTer22) in 2 families. These variants were already reported in the literature, as well as the variants c.286 C > T; p.(Arg96Ter), c821_825dup; p.(Asn276GlnfsTer61) and c.1473_1474del; p.(Gly493ThrfsTer21). All missense variants

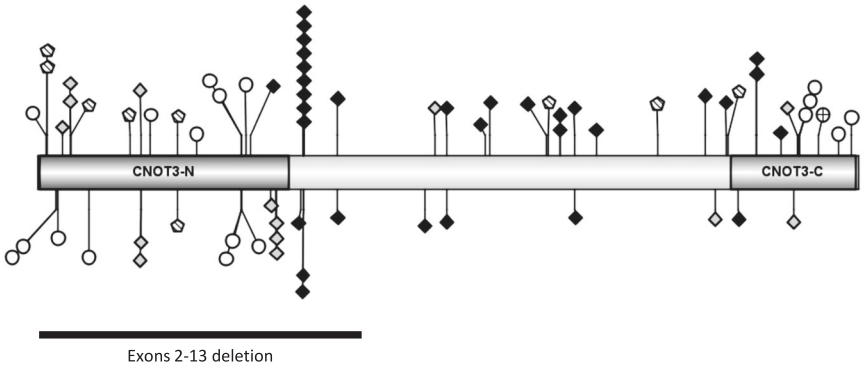

Fig. 3 Graphical representation of *CNOT3* variants (IBS 1.0.3). Variants identified in our cohort are above, literature variants are below. Each circle represents a patient with a missense variant, the one with a cross represents a patient with an inframe deletion. Diamonds represent patients with truncating variants: nonsense are in gray and frameshift in black. Hatched pentagons represent splice variants. The black line represents the deletion of 12 exons. Note that all the missense variants are on both extremities of the protein and frameshift variants in the median part.

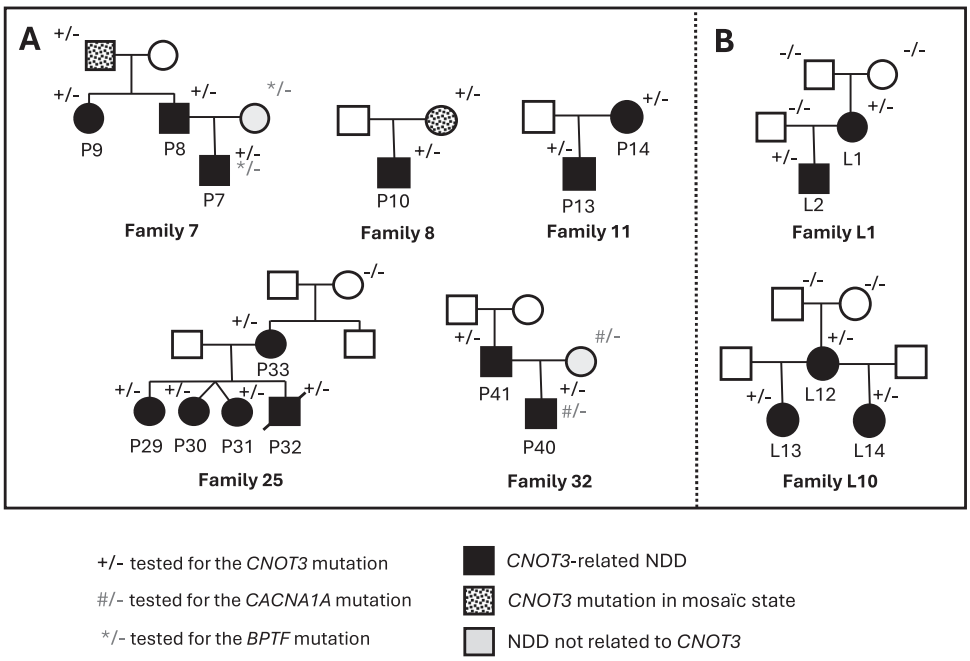

Fig. 4 Pedigrees of familial cases. Pedigrees of familial cases from our cohort (**A**) and the literature (**B**). A second variant in a gene involved in NDD was identified in families 7 and 32 and was inherited from the other symptomatic parent. Two parents carry the variant found in their children in a mosaic state (Families 7 and 8).

identified in this study were classified as probably or possibly damaging by CADD, PolyPhen-2, and SIFT prediction tools (Supplementary data S3), except for the two variants c.25 G > C; p.(Gly9Arg) and c.2090 G > A; p.(Arg697Gln) for which bioinformatic results were non-consistent. None of the missense variants were present in the gnomAD database (v4.1.0) [18]. The c.25 G > C; p.(Gly9Arg) variant, affecting the last nucleotide of exon 2, was predicted to alter splicing.

For 35 individuals, the variant occurred de novo, while it was inherited from a symptomatic parent for 7 patients, including 4 children from the same family (Fig. 4). For three individuals from 2 families (Families 7 and 8), the variant was present in a mosaic state in an unaffected parent.

In three patients, an additional LP/P variant in another gene was identified, namely a de novo variant in *TNPO3* involved in variable limb-girdle muscular dystrophy (LGMD) in patient P6; a variant in *BPTF*, involved in NDD, inherited from a symptomatic mother in patient P7; and a variant in *CACNA1A*, involved in developmental and epileptic encephalopathy, maternally inherited, in patient P40. In the literature, a variation in *SMAD6*, involved in aortic valve disease, was identified in patient L16, reported by Priolo et al. [5]. It remains difficult to determine whether clinical manifestations are linked to *CNOT3* or to the additional LP/P variant in the second gene, or if patients with two variants are more severe due to the interaction of genetic and environmental factors. Moreover, the presence of a *SMAD6* variation in patient L16 certainly explains his aortic stenotic coarctation (CoA), hypertrophic left ventricle and bicuspid aortic valve (BAV). At the time of examination, patient P7 had no signs of muscular dystrophy.

DISCUSSION

Thanks to an in-depth characterization of a large cohort of 51 new individuals, we were able to further delineate the spectrum of

CNOT3-related neurodevelopmental disorders *(CNOT3*-NDD*)*. Overall, the clinical picture seems variable, and no case of non-penetrance was shown in the cohort. Developmental delay was present in all individuals, but with speech therapy, 84% of individuals acquired the ability to speak at least a few words. Intellectual disability was mild to moderate in the vast majority of cases (94%). Behavioral difficulties and autism spectrum disorders affected more than half of the individuals. Interestingly, we were able to describe 10 adult individuals, who showed the ability to integrate into society with sheltered employments. Long-term follow-up of these adult individuals will be of major importance in determining the natural history of the condition. In keeping with the literature, the occurrence of epilepsy in our series was infrequent (5/51; 10%). Congenital birth defects were rare and varied. We noticed a relatively large number of cases of inguinal or umbilical hernia. Common dysmorphic features that may help make the diagnosis were a high forehead, upslanting palpebral fissures, a low hanging columella, hypoplastic alae nasi and a thin upper lip. Interestingly, short stature reported in the initial articles [2, 3] was only documented in one quarter of our patients. Moreover, the association with Moya Moya disease suggested by Pinard et al. [4]. was not confirmed in our cohort. It should be noted that of the three variants reported to be associated with this condition, two were identified in individuals with no neurodevelopmental disorders. In one case, the variant was inherited from an asymptomatic father. However, no MRA-TOF was performed in our patients, so we cannot rule out an association between Moya Moya disease and certain *CNOT3* variants.

Study of genotype-phenotype correlations showed no significant differences or distinctive features between individuals with different types of variants in the *CNOT3* gene. We did not observe any difference in the severity of ID between the different groups. Mild ID appears to be more frequent than moderate ID in patients with a missense variant (6/9; 67% and 1/9; 11% respectively) than in those with a truncating variant (12/20; 60% and 8/20; 40% respectively) or a splice variant (1/4; 25% and 3/4; 75% respectively). However, the five individuals without ID had truncating or splice variants and both individuals with severe ID carried missense variants. This absence of correlation is corroborated by the fact that the rate of individuals admitted to special-need schools is high in the three groups (8/10; 80%, 20/28; 71% and 5/6; 83% respectively). Furthermore, in the familial cases of our cohort, the degree of intellectual disability varied between affected members of the same family, which is consistent with the absence of genotype-phenotype correlation for ID in this gene.

In two inherited cases from the cohort (Families 7 and 32), the *CNOT3*-NDD occurred in an environment where a pathogenic variant in a NDD-gene segregates in the other family branch (Fig. 4, Supplementary Data S1 and Supplementary Data S5). Double diagnosis is estimated to occur in around 3.5% of NDD cases [28] and this phenomenon could be more common in children of couples where both members are intellectually disabled. A second diagnosis was not identified by exome sequencing in any of the two patients with severe ID, and it is therefore not possible to conclude at present whether there is a second acquired or genetic cause that would explain the severity, or whether this is simply the extremity of *CNOT3*-NDD clinical spectrum. In the literature, some reported patients have no language [2] which could suggest the existence of severe forms, but the data are insufficient to reach a conclusion. Epilepsy seemed to be more common in patients with a missense variant in the N-terminal domain in our cohort (4 of 5 patients), but this was not the case for patients from the literature. Three of eight unrelated individuals with epilepsy shared the same c.563 G > A; p.(Arg188His) variant. We suggest that a different impact of this variant, such as a gain-of-function effect, could possibly explain this difference. DynaMut2 [29] predicts a destabilizing change for this variant but further functional studies are required to verify this hypothesis. It should also be noted that several patients had suspected seizures without a proven diagnosis, indicating that a better neurological evaluation would be important to truly understand the prevalence of epilepsy in this condition.

We noticed that nonsense and missense variants are almost exclusively located in the N- and C- terminal parts of the protein, which are considered as domains [30, 31], while frameshifts are rather located in the median part of *CNOT3*. The higher frequency of frameshift variants in this segment could be linked to the higher proportion of cytosine-rich sites in this region. Interestingly, there are only two *CNOT3* truncating variants reported in gnomAD genomes v4.1.0 for the MANE reference transcript (ENST00000221232.11—NM_014516.4) [18] and none in the predicted nonsense-mediated decay (NMD) escape region. As no truncating variant in this area was reported in gnomAD, we can suppose that variants in this region could also be deleterious for the protein.

The high occurrence of missense variants at both ends of the protein could probably be explained by the function of CNOT3 itself. Structurally, the CCR4-NOT complex is L-shaped and consists of a catalytic module comprising two deadenylases and a NOT-module formed by the CNOT1, CNOT2 and CNOT3 subunits in humans [30, 31]. CNOT3 binds to CNOT2 and CNOT1 via the NOT-Box domain in its C-terminal region [30]. When exposed to non-optimal mRNA, CNOT3 binds to the ribosomal E-site via its N-terminal part and permits recruitment of the CCR4-NOT complex via its C-terminal domain, leading to decapping of non-optimal mRNA by deadenylases [31, 32]. The occurrence of missense variants only in these two regions seems consistent with the loss-of-function mechanism initially suggested in the literature [1]. In fact, as these are located in binding domains, their effect could be null and equivalent to truncating variants. This might explain the absence of phenotypic differences between individuals according to the type of variants identified.

Presence of pathogenic missense variants elsewhere than in these functional domains is unlikely, given the number of missense variants reported in gnomAD and a landscape that appears to be tolerant in MetaDome [33]. We know that missense variants in the middle third of the protein were reported by Pinard et al. in individuals with Moya Moya disease but no neurodevelopmental disorder [4]. We did not consider these variants in our cohort, as they are not considered as pathogenic by ACMG classification nor by in silico predictions tools. However, we cannot exclude the possibility of any effect other than loss-of-function of these variants.

Interestingly, parental gonosomal mosaicism was reported in 2 of the 42 families of our cohort. Considering that parental segregation could not be performed in 6 families, and the unknown rate of germline mosaicism, the recurrence risk for de novo *CNOT3* mutations could be higher than 1%.

Overall, the core clinical phenotype associated with pathogenic or likely pathogenic *CNOT3* variants includes developmental delay, mild to moderate intellectual disability, dysmorphism, behavioral disorders and autistic features. The absence of a clear genotype-phenotype correlation may be attributed to the uniform loss-of-function impact of all identified variants. Other studies are necessary to further describe the adult phenotype, and better understand the functional consequences of the variants.

DATA AVAILABILITY

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request. Pathogenic and probably pathogenic variants in our cohort that had not previously been reported were submitted to ClinVar (Supplementary Data S3).

REFERENCES

1. Collart MA. The Ccr4-Not complex is a key regulator of eukaryotic gene expression. Wiley Interdiscip Rev RNA. 2016;7:438–54.
2. Martin R, Splitt M, Genevieve D, Aten E, Collins A, de Bie CI, et al. De novo variants in CNOT3 cause a variable neurodevelopmental disorder. Eur J Hum Genet. 2019;27:1677–82.
3. Meyer R, Begemann M, Demuth S, Kraft F, Dey D, Schüler H, et al. Inherited cases of CNOT3-associated intellectual developmental disorder with speech delay, autism, and dysmorphic facies. Clin Genet. 2020;98:408–12.
4. Pinard A, Guey S, Guo D, Cecchi AC, Kharas N, Wallace S, et al. The pleiotropy associated with de novo variants in CHD4, CNOT3, and SETD5 extends to moyamoya angiopathy. Genet Med J Am Coll Med Genet. 2020;22:427–31.
5. Priolo M, Radio FC, Pizzi S, Pintomalli L, Pantaleoni F, Mancini C, et al. Co-Occurring Heterozygous CNOT3 and SMAD6 Truncating Variants: Unusual Presentation and Refinement of the IDDSADF Phenotype. Genes. 2021;12:1009
6. Lv J, Ming WJ, Zheng Y, Xu S, Fang GL, Zhang Q, et al. A novel pathogenic variant in CNOT3 causing neurodevelopmental delay and epilepsy. Seizure. 2023;107:104–6.
7. Zhao P, Meng Q, Wan C, Lei T, Zhang L, Zhang X, et al. Clinical features of CNOT3-associated neurodevelopmental disorder in three Chinese patients. Neurogenet. 2023;24:129–36.
8. Lee CG, Kim HJ, Seol CA, Ki CS. Novel in-frame deletion CNOT3 variant in a family with intellectual developmental disorder with speech delay and dysmorphic facies. Neurol Genet. 2024;10:e200116.
9. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36:928–30.
10. DECIPHER: database for the interpretation of phenotype-linked plausibly pathogenic sequence and copy-number variation - PMC [Internet]. [cité 1 sept 2023]. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3965078/.
11. Baux D, Van Goethem C, Ardouin O, Guignard T, Bergougnoux A, Koenig M, et al. MobiDetails: online DNA variants interpretation. Eur J Hum Genet EJHG. 2021;29:356–60.
12. Rentzsch P, Witten D, Cooper GM, Shendure J, Kircher M. CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. 2019;47:D886–94.
13. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010;7:248–9.
14. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res. 2012;40:W452–7.
15. Landrum MJ, Lee JM, Benson M, Brown GR, Chao C, Chitipiralla S, et al. ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res. 2018;46:D1062–7.
16. Fokkema IFAC, den Dunnen JT, Taschner PEM. LOVD: easy creation of a locus-specific sequence variation database using an « LSDB-in-a-box » approach. Hum Mutat. 2005;26:63–8.
17. Day INM. dbSNP in the detail and copy number complexities. Hum Mutat. 2010;31:2–4.
18. Gudmundsson S, Singer-Berk M, Watts NA, Phu W, Goodrich JK, Solomonson M, et al. Variant interpretation using population databases: Lessons from gnomAD. Hum Mutat. 2022;43:1012–30.
19. Nassar LR, Barber GP, Benet-Pagès A, Casper J, Clawson H, Diekhans M, et al. The UCSC Genome Browser database: 2023 update. Nucleic Acids Res. 2022;51:D1188–95.
20. Davydov EV, Goode DL, Sirota M, Cooper GM, Sidow A, Batzoglou S. Identifying a high fraction of the human genome to be under selective constraint using GERP++. PLoS Comput Biol. 2010;6:e1001025.
21. Grantham R. Amino acid difference formula to help explain protein evolution. Science. 1974;185:862–4.
22. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. 2015;31:2745–7.
23. Leman R, Parfait B, Vidaud D, Girodon E, Pacot L, Le Gac G, et al. SPiP: Splicing Prediction Pipeline, a machine learning tool for massive detection of exonic and intronic variant effects on mRNA splicing. Hum Mutat. 2022;43:2308–23.
24. 罕见病数据库 [Internet]. [cité 2 févr 2025]. Disponible sur: https://rddc.tsinghua-gd.org/tool/rna-splicer?id=4849.
25. de Sainte Agathe JM, Filser M, Isidor B, Besnard T, Gueguen P, Perrin A, et al. SpliceAI-visual: a free online tool to improve SpliceAI splicing variant interpretation. Hum Genomics. 2023;17:7.
26. Liu W, Xie Y, Ma J, Luo X, Nie P, Zuo Z, et al. IBS: an illustrator for the presentation and visualization of biological sequences. Bioinformatics. 2015;31:3359–61.
27. on behalf of the ACMG Laboratory Quality Assurance Committee, Richards S, Aziz N, Bale S, Bick D, Das S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405–23.
28. Franklin by Genoox: The Future of Genomic Medicine. 2022. [Internet]. 2022. Disponible sur: https://franklin.genoox.com.
29. Rodrigues CHM, Pires DEV, Ascher DB. DynaMut2: assessing changes in stability and flexibility upon single and multiple point missense mutations. Protein Sci Publ Protein Soc. 2021;30:60–9.
30. Boland A, Chen Y, Raisch T, Jonas S, Kuzuoğlu-Öztürk D, Wohlbold L, et al. Structure and assembly of the NOT module of the human CCR4-NOT complex. Nat Struct Mol Biol. 2013;20:1289–97.
31. Collart MA, Panasenko OO, Nikolaev SI. The Not3/5 subunit of the Ccr4-Not complex: A central regulator of gene expression that integrates signals between the cytoplasm and the nucleus in eukaryotic cells. Cell Signal. 2013;25:743–51.
32. Absmeier E, Chandrasekaran V, O'Reilly FJ, Stowell JA, Rappsilber J, Passmore LA. Specific recognition and ubiquitination of translating ribosomes by mammalian CCR4-NOT. Nat Struct Mol Biol. 2023;30:1314.
33. Wiel L, Baakman C, Gilissen D, Veltman JA, Vriend G, Gilissen C. MetaDome: Pathogenicity analysis of genetic variants through aggregation of homologous human protein domains. Hum Mutat. 2019;40:1030–8.

AUTHOR CONTRIBUTIONS

CE, MR, JA collected clinical and molecular data. CE, JP analyzed and interpreted data and wrote the manuscript. JP designed the study. EA, FA, ALAD, EKB, PB, LPB, BC, VC, MC, BC, SC, CC, BD, ED, ADD, ASDP, BD, LD, JF, DG, TG, MMH, DH, IH, MI, BI, BK, SK, DAK, AK, JL, CL, JL, CL, SM, SM, CM, GM, IN, SN, LN, EP, AP, JP, JP, FP, AR, AR, MR, AR, LR, MS, JS, EHS, AS, RS, TS, JS, PS, MS, HS, FTMT, AMT, JVG, GV, JJAV, CV, CVD, EV, EW, PZ, FZ, PK, JP characterized the clinical and molecular features of the disease. All the authors edited or commented the manuscript.

FUNDING

Research reported in this publication was supported by Jordan's Guardian Angels, the Sunderland Foundation and the Brotman Baty Institute (to G.M.M.). E.W. : The DDD study presents independent research commissioned by the Health Innovation Challenge Fund [grant number HICF-1009-003]. This study makes use of DECIPHER (http://www.deciphergenomics.org), which is funded by Wellcome [grant number WT223718/Z/21/Z]. See Nature PMID: 25533962 or www.ddduk.org/access.html for full acknowledgement. P.Z.'s work is being supported by Stiftung Michael through the generous assistance of the Canger-Janz-Fellowship. L.N. was supported by the project National Institute for Neurological Research (Programme EXCELES, ID Project No. LX22NPO5107) - Funded by the E'uropean Union – Next Generation EU. L.N. and S.K. were supported by grant NU23-07-00281 from the Ministry of Health of the Czech Republic, by the institutional program UNCE/24/MED/022 of Charles University in Prague and they thank to the National Center for Medical Genomics (LM2023067) for WES analyses.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL

Individuals were referred by clinical geneticists for genetic testing as part of routine clinical care. All patients enrolled and/or their legal representative have signed informed consent for use of data and/or photographs.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41431-025-01884-z.

Correspondence and requests for materials should be addressed to Camille Engel or Juliette Piard.

[1]Université de Franche-Comté, Centre de Génétique Humaine, CHU Besançon, Besançon, France. [2]Université de Bourgogne, INSERM UMR1231 GAD "Génétique des Anomalies du Développement", Dijon, France. [3]Department of Neurology, University of California, San Francisco, San Francisco, CA, USA. [4]Medical Genetics, University of Siena, Siena, Italy. [5]Med Biotech Hub and Competence Center, Department of Medical Biotechnologies, University of Siena, Siena, Italy. [6]Genetica Medica, Azienda Ospedaliera Universitaria Senese, Siena, Italy. [7]Department of Clinical Genetics, Leiden University Medical Centre, Leiden, The Netherlands. [8]Laboratoire Seqoia, Paris, France. [9]Department of Medicine and Surgery, University of Milan-Bicocca, Monza, Italy. [10]Reference centre for Mendelian connective tissue disorders - UZ Gent, Ghent University Hospital—UZ Gent, Gent, Belgium. [11]Genomics and Clinical Genetics, IRCCS Istituto Giannina Gaslini, Genoa, Italy. [12]Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Roma, Italy. [13]Service de Génétique Médicale, Hôpital Purpan, CHU de Toulouse, Toulouse, France. [14]Center for Medical Genetics, Hôpital Erasme, Hôpital Universitaire de Bruxelles, Université Libre de Bruxelles, Brussels, Belgium. [15]Genetic Medicine, University of California, San Francisco/Fresno, Fresno, CA, USA. [16]Genetics and Metabolics Clinic, McMaster Children's Hospital, Hamilton, ON, Canada. [17]Université de Franche-Comté, CHU Besançon, Oncobiologie Génétique Bioinformatique, FHU-TRANSLAD et Institut GIMI, Besançon, France. [18]UF médecine génomique et génétique clinique, Hôpital Jean Verdier, Hôpitaux Universitaires de Paris Seine Saint Denis, AP-HP, Bondy, France. [19]UFR de Santé Médecine et Biologie humaine, Université Sorbonne Paris Nord, Bobigny, France. [20]NeuroDiderot UMR 1141, Inserm, FHU I2-D2, Université de Paris, Paris, France. [21]Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France. [22]Service de Génétique Clinique et Oncogénétique, CHU Amiens-Picardie, Amiens, France. [23]Department of Biology and Medical Genetics, Charles University—2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic. [24]Institute for Clinical Genetics, University Hospital Carl Gustav Carus at TUD Dresden University of Technology, Dresden, Germany. [25]Université Montpellier, Centre de référence anomalies du développement et syndromes malformatifs, Génétique Clinique, CHU Montpellier, Montpellier, France. [26]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy. [27]Child Neuropsychiatry Unit, IRCCS G. Gaslini Institute, Genoa, Italy. [28]Center for Rare Diseases, Pediatric and Adolescent Medicine, Aarhus University Hospital, Aarhus, Denmark. [29]Département de génétique médicale, Hôpital Pitié-Salpêtrière, AP-HP.Sorbonne Université, Paris, France. [30]Institute of Human Genetics, University Hospital Schleswig-Holstein, Lübeck, Germany. [31]Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy. [32]Service de génétique médicale, Unité de génétique clinique, CHU Hôtel Dieu, 1, place Alexis Ricordeau, Nantes, France. [33]Research Unit for Rare Diseases, Department of Pediatrics and Inherited Metabolic Disorders, 1st Faculty of Medicine, Charles University in Prague, Prague, Czech Republic. [34]Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands. [35]Department of Medical Genetics, Masaryk Hospital in Ústí nad Labem, Regional Health Corporation, Ústí nad Labem, Czech Republic. [36]Genetics Department, AP-HP, Robert-Debré University Hospital, Paris, France. [37]Service of Medical Genetics, Oncologic Institute of Southern Switzerland, EOC, Switzerland, Switzerland. [38]Centre de référence « Surdités Génétiques », Service de Médecine génomique des Maladies rares, Hôpital Necker, Assistance Publique - Hôpitaux de Paris, Paris, France. [39]Institut Imagine, Paris, France. [40]Department of Pediatrics, University of Washington, Seattle, WA, USA. [41]Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA. [42]Brotman Baty Institute for Precision Medicine, Seattle, WA, USA. [43]Institute of Human Genetics, University Hospital Schleswig-Holstein, Kiel, Germany. [44]Department of Genetics, Hôpital Universitaire des Enfants Reine Fabiola, Hôpital Universitaire de Bruxelles, Université Libre de Bruxelles, Brussels, Belgium. [45]Department of Neurobiology and Molecular Medicine, IRCCS Fondazione Stella Maris, Pisa, Italy. [46]Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg, 91054 Erlangen, Germany. [47]Centre for Rare Diseases Erlangen (ZSEER), Erlangen, Germany. [48]Necker Hospital, APHP, Reference Center for Intellectual Disability, Genetics Department, Institut Imagine, University of Paris, Paris, France. [49]Division of Human Genetics, Children's Hospital of Philadelphia, Philadelphia, PA, USA. [50]Radboud University Medical Center, Department of Neurology, Nijmegen, The Netherlands. [51]Department of Pediatrics, Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada. [52]Department of Medical Genetics and Metabolism, Valley Children's Hospital, Madera, CA, USA. [53]Institut de Génétique Médicale, CHU Lille, Avenue Oscar Lambret, Lille, France. [54]Interuniversitary Institute of Bioinformatics in Brussels, Université Libre de Bruxelles, Brussels, Belgium. [55]Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini, Genova, Italy. [56]Nottingham Clinical Genetics Service, Nottingham University Hospitals NHS Trust, City Hospital Campus, Nottingham, UK. [57]Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium. [58]Department of biomolecular medicine, Ghent university, Ghent, Belgium. [59]Department of Medical Genetics, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal. [60]Institute of Histology and Developmental Biology, Faculty of Medicine, University of Lisbon, Lisbon, Portugal. [61]Department of Medical Genetics, University Hospital of Bordeaux and INSERM U1211, University of Bordeaux, Bordeaux, France. [62]CHU Lille, Clinique de Génétique, Lille, France. [63]North East Thames Regional Genetic Service, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK. [64]Epilepsy Center Kleinwachau, Radeberg, Germany.