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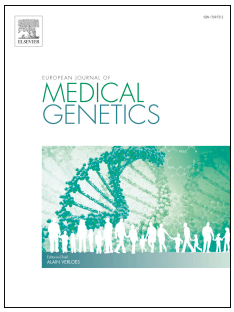

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Penetrance, Variable Expressivity and Monogenic Neurodevelopmental Disorders

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ABSTRACT

Purpose: Incomplete penetrance is observed for most monogenic diseases. However, for neurodevelopmental disorders, the interpretation of single and multi-nucleotide variants (SNV/MNVs) is usually based on the paradigm of complete penetrance.

Method: From 2020 to 2022, we proposed a collaboration study with the French molecular diagnosis for intellectual disability network. The aim was to recruit families for whom the index case, diagnosed with a neurodevelopmental disorder, was carrying a pathogenic or likely pathogenic variant for an OMIM morbid gene and inherited from an asymptomatic parent. Grandparents were analyzed when available for segregation study.

Results: We identified 12 patients affected by a monogenic neurodevelopmental disorder caused by likely pathogenic or pathogenic variant (SNV/MNV) inherited from an asymptomatic parent. These genes were usually associated with *de novo* variants. The patients carried different variants (1 splice-site variant, 4 nonsense and 7 frameshift) in 11 genes: *CAMTA1, MBD5, KMT2C, KMT2E, ZMIZ1, MN1, NDUFB11, CUL3, MED13, ARID2* and *RERE*. Grandparents have been tested in 6 families, and each time the variant was confirmed *de novo* in the healthy carrier parent.

Conclusion: Incomplete penetrance for SNV and MNV in neurodevelopmental disorders might be more frequent than previously thought. This point is crucial to consider for interpretation of variants, family investigation, genetic counseling, and prenatal diagnosis. Molecular mechanisms underlying this incomplete penetrance still need to be identified.

Keywords: neurodevelopmental disorder, intellectual disability, incomplete penetrance, monogenic

INTRODUCTION

Penetrance is defined as the percentage of individuals having a disease-causing genotype who exhibit phenotypic features(Cooper et al., 2013). The association of pathogenic variants with phenotypes such as intellectual disability has been almost exclusively evaluated using clinically ascertained cohorts, which might explain an often-overestimated penetrance. In neurodevelopmental disorders, variant interpretation is most frequently based on the paradigm of complete penetrance, with sporadic cases considered to be mostly caused by *de novo* variants (Hamdan et al., 2017; O'Roak et al., 2011; Topper et al., 2011; Vissers et al., 2010). Therefore, trio-exome or genome analysis, which helps filter *de novo* variants, is an essential tool for the genetic diagnosis of intellectual disability and is often used as a first-line strategy in sporadic cases for neurodevelopmental disorders (Gilissen et al., 2014; Wright et al., 2018). However, incomplete penetrance has been observed for neurodevelopmental disorders, mostly for recurrent Copy Number Variant (CNV) involving several genes (Kendall et al., 2019; Männik et al., 2015). There are less data concerning SNV, but a recent study has shown that rare, potentially damaging variants in genes and *loci* linked to dominant monogenic developmental disorders are present in adults in clinically unselected population cohort (UK Biobank) and result in a mild developmental phenotype (Kingdom et al., 2022).

In this study, we present 12 patients affected by a neurodevelopmental disorder, carrying a pathogenic or likely pathogenic loss-of-function variant in a gene known to cause a neurodevelopmental phenotype with a high penetrance inherited from a parent considered as asymptomatic.

METHODS

From 2020 to 2022, 12 patients were recruited from 5 University Hospital Centers in France (Nantes, Rouen, Besançon, Poitiers, Dijon) through French molecular diagnosis for intellectual disability network. The referring clinicians and biologists sent the clinical and biological information. Selected patients showed a neurodevelopmental disorder, and carried an inherited variant classified as

pathogenic or likely pathogenic (class 4/5 by the American College of Medical Genetics and Genomics (ACMG; (Richards et al., 2015)) explaining the phenotype and compatible with the known molecular mechanism, identified through large panel or exome sequencing. The carrier parent had to be reported as asymptomatic by the referring clinician at the time of the analysis. Neuropsychological evaluation was not performed for parents. Grandparents were tested for the variant when available by cascade testing (Sanger). Patients signed a written informed consent for the use of their medical history and genetic testing report.

RESULTS

Clinical cohort

We identified 12 patients (5 females and 7 males), born between 1978 and 2015, carrying a pathogenic or likely pathogenic variant in a known neurodevelopmental disorder gene (**Table 1**). Neurobehavioral phenotype showed one patient with a specific learning disability, while the rest of the cohort had a variable degree of intellectual disability. In addition, 8/12 had a behavioral disorder. About clinical features, 7/12 showed facial dysmorphism and 5/12 had at least one congenital malformation. Patients were referred between the age of 13 months and 27 years old.

Molecular analysis

The 12 pathogenic or probably pathogenic variants were identified in 11 genes, all known to cause neurodevelopmental disorders referenced in OMIM: *CAMTA1* [Cerebellar dysfunction with variable cognitive and behavioral abnormalities MIM614756], *MBD5* [Intellectual developmental disorder, autosomal dominant 1 MIM156200], *KMT2C* [Kleefstra syndrome 2 MIM617768], *ZMIZ1* [Neurodevelopmental disorder with dysmorphic facies and distal skeletal anomalies MIM618659], *MN1* [CEBALID syndrome 618774], *NDUFB11* [Linear skin defects with multiple congenital anomalies 3 MIM300952], *CUL3* [Neurodevelopmental disorder with or without autism or seizures MIM619239], *MED13* [Intellectual developmental disorder, autosomal dominant 61 MIM618009],

ARID2 [Coffin-Siris syndrome 6 MIM617808], *RERE* [Neurodevelopmental disorder with or without anomalies of the brain, eye, or heart MIM616975] and *KMT2E* [O'Donnell-Luria-Rodan syndrome MIM618512] which was mutated in two patients **(Table 1)**. These variants were classified as likely pathogenic or pathogenic according to the criteria of the ACMG criteria. The individual criteria supporting each variant classification are detailed in Table 1.

All variants were localized in genes with a high probability of Loss of function Intolerance (gnomAD v4, Probability of being loss-of-function intolerant (pLI), threshold ≥0.90; observed/expected (o/e) ratio= 0.03 ([0.01-0.16] 90% CI, threshold <0.35)), showing a very high constraint on loss-of function variant, with all genes having a very low o/e score, defining a very strong intolerance to such variants **(Table 1)**.

Of note, although *NDUFB11* has the lowest pLI (0.8) just below the recommended threshold, the o/e ratio is very low (0; 0-0.56 confidence interval), and the only loss of function variant found in gnomAD was attached to multiple warnings regarding quality metrics and coverage (artefactual finding, which can be verified by visualizing the alignment data for the variant X-47003870-A-C; gnomAD website).

All truncating variants were located outside the last exon, except for patient 5 carrying a frameshift variant in the last exon of *KMT2E*. For patient 7, the *MN1* variant was located in the only exon, and was predicted to escape nonsense-mediated mRNA decay, with a dominant negative effect as previously described (Mak et al., 2020).

In addition, a mRNA analysis was performed in patient 9 carrying a splice-site variant, which showed a complete exon skipping inducing a premature stop codon (supplemental data).

Two patients carried variants already reported as pathogenic in previous publications: patient 3 (Faundes et al., 2017) and patient 7 (Mak et al., 2020).

All patients had clinical features compatible with their molecular diagnosis. Moreover, patient 7 and 8 had a specific phenotype evocative of respectively MCTT syndrome (caused by *MN1* C-terminal

truncating variants) and Linear skin defects with multiple congenital anomalies (caused by *NDUFB11* pathogenic variants). For both patients, the variant was inherited from a parent with no clinical (neurological, dermatological, morphological) symptom.

Looking at inheritance pattern, 6/12 variants were inherited from the father and 6/12 from the mother **(Figure 1)**. Mosaicism was not observed in our patients or their parents, in the blood and/or other tissues when available (DNA from saliva or skin were tested in 3/12 carrier parents). Each time grand-parents could be tested (6 families), the variant was confirmed to be *de novo* in the healthy carrier parent, adding to the list of molecular arguments (Daum et al., 2020).

DISCUSSION

We identified 12 individuals affected by neurodevelopmental disorders caused by a likely pathogenic or pathogenic single or multi-nucleotide variant in 11 different genes, inherited from an asymptomatic parent.

To our knowledge, there is no notion of incomplete penetrance reported for *KMT2C, KMT2E, MN1, CUL3, MED13, ARID2* and *MBD5*. For *CAMTA1*, Jacobs et al. described one variant inherited from an apparently healthy mother in a cohort of 19 patients (Jacobs et al., 2020). For *NDUFB11*, two other patients were published by van Rahden *et al.*, where one variant was inherited from an asymptomatic mother (van Rahden et al., 2015). Finally, for *ZMIZ1*, Latchman *et al.* described a family with one father and two children who had the same variant, but showed an important phenotypic variability (Latchman et al., 2019). Likewise, pathogenic variants in *RERE* in a mother and her child, with a fairly different phenotype have been described recently (Niehaus et al., 2022).

These molecular results and the associated parental segregation data were unexpected by the clinical geneticist following the families, as the parents did not show similar clinical features to their consulting child. To date, we have not been able to identify or hypothesize any clear environmental or molecular contributor to the incomplete penetrance.

This observation has several implications. First, it is now necessary to integrate the notion of incomplete penetrance in the method of variants interpretation and to consider inherited variants with great caution. SNVs and MNVs inherited from healthy parents should not be systematically classified as likely benign and should even be considered as pathogenic if clinical and molecular arguments converge. The current interpretation of pathogenicity is often easier for variants associated with loss of function, which probably explains that we only retain these variants in our cases. It is likely that some inherited missense variants are also pathogenic, which is often more difficult to prove. This also impacts the familial investigation, therefore segregation studies should be systematically proposed to know the molecular status of the parents, even if they are described as asymptomatic. Regarding genetic counselling, these penetrance defects complicate predictions, especially in prenatal diagnosis: a parent carrying a variant may not be sufficient to be fully reassuring and conversely, a predicted pathogenic mutation detected in a fetus may not generate a severe phenotype.

One of the limitations of this study is that parents have been considered as asymptomatic without any objectified or quantifiable neurological evaluation, therefore we cannot affirm that they have no subtle clinical manifestations that were not detected by the referring clinician. We retained the parents initially considered as unaffected by the clinician following the first consultation. Most of these parents reported a classical school cursus and no clinical signs related to the identified variant, and none of them declared any neurological symptom. They did not present any dermatological or dysmorphic feature when these were classically described in the identified syndrome but no formal cognitive evaluation has been performed. A study on copy number variants responsible for schizophrenia and autism spectrum disorders showed that "control" carriers considered healthy tend to show lower cognitive abilities or verbal fluency tests (Männik et al., 2015; Stefansson et al., 2013). Thus, it is now admitted that these CNV have an impact in carrier parents, which could be undetected clinically without specific explorations like standard neuropsychological testing. This could also be true for the SNVs and MNVs reported here, but we still lack statistical power to test this hypothesis.

It might also be even more relevant and interesting to compare the results of these tests in parents to the grand-parents, rather than with the affected child. Still, affected children were most often severely affected, in contrast with the absence of any reported symptoms or reported clinical features for the carrier parent.

To date, molecular mechanisms explaining this incomplete penetrance remain unknown. Several hypotheses are described to explain variable expressivity within a family (Cooper et al., 2013). First, there is the possibility of mosaicism, which was not observed in the parents for the available samples (*KMT2C, ZMIZ1, MN1*). Though, when grand-parents were available for testing (5/12), the variants always were *de novo* in the healthy parent (*CAMTA1, MBD5, KMT2C, MN1, RERE*). We can hypothesize that, in neurodevelopmental disorders, we could miss mosaicism by studying blood samples, especially if variants give a selective advantage during hematopoiesis as it is well known for *ASXL1* (Carlston et al., 2017) or *DNMT3A* (Jaiswal et al., 2014). Parental imprinting could also be discussed as a modulatory mechanism, but none of these 11 genes are known to be subject to parental imprinting. Gender could also influence the phenotype, but our cases include 7 males and 5 females. X-inactivation could explain the case of patient 8 since *NDUFB11* is located on the X chromosome, unfortunately this could not be performed in the mother. Though X-inactivation could explain some of the phenotypic variability, it might not be the only mechanism explaining the variable expressivity for X-linked disorder (Sun et al., 2022).

Another explanation could be a phenomenon called “multi-hit” where a second variant in the genome could modify the phenotypic expression caused by the first pathogenic variant (Girirajan et al., 2010; Posey et al., 2017). Yet, the phenotypes of the probands were in line with previous reports and no other pathogenic variant (SNV/MNVs and CNVs) could be identified by array comparative genomic hybridization (array CGH) or exome sequencing. Genetic compensation -also called transcriptional adaptation (El-Brolosy et al., 2019; Ma et al., 2019)-, allelic imbalance and epigenetic factors could also explain incomplete penetrance (Bray et al., 2003; Pastinen et al., 2004; Wilkinson, 2019), though

our patients could not benefit from such functional testing. Finally, this work does not provide any clear molecular mechanisms underlying this incomplete penetrance. This point is crucial to consider for interpretation of variants, family investigation, genetic counseling, and prenatal diagnosis. Yet, as we often do not have access to a functional study for every variant identified, our interpretation is frequently restricted to the segregation study, which is probably too simplistic... As the worldwide use of genome sequencing as a first-tier diagnosis is very rapidly increasing and now proposed to be used for asymptomatic children as a neonatal screening test, a very rapid progress for data interpretation will also be the major ongoing challenge for the next decade. Further molecular investigations and research will be essential to develop our understanding of the mechanism i penetrance in humans, which could alsogive clue for future molecular therapies..

DATA AVAILABILITY

All data are available upon request.

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AUTHOR CONTRIBUTION STATEMENT

Conceptualization: SDM, BI

Data curation: All authors

Methodology: SDM, BI

Writing – original draft: SDM, BI

Writing – review & editing: All authors

ETHICAL APPROVAL

Written informed consent was obtained for the use of medical history and genetic testing report, as approved by the Institutional Review Board of the University Hospital Center (CHU) of Nantes.

COMPETING INTERESTS

All authors declare no conflict of interest.

TABLES

Table 1 : Detailed clinical features of the patients.

FIGURES

Figure 1 : Pedigree of the 12 families.

SUPPLEMENTARY INFORMATION

CUL3 mRNA analysis

Detailed clinical features of the patients

Patient 1 is the first girl of two children born to non-consanguineous parents. Family history was unremarkable other than a slight language delay in the father. Her father is a technical draftsman and had some academic difficulties in college, yet he obtained a professional license. Following an uncomplicated pregnancy, the proband was born at 36 weeks of gestation with normal measurements. Development was delayed: she walked at two years old, and her first words were pronounced after three years old. Autism Diagnostic Observation Schedule at 6 years old revealed an autism spectrum disorder. She showed a learning disability. At 9 years and 11 months, her height, weight and head circumference (HC) were 135 cm (M), 26,7 kg (-0,5 Standard deviation (SD)) and 51 cm (-1 SD), respectively. No dysmorphic features were noted except for a discrete hypertrichosis of the elbow. She also showed significant velar insufficiency.

Previous testing included a normal chromosomal microarray. ES identified a pathogenic variant in *CAMTA1* c.2871_2883del, p.(Thr959Profs*31) inherited from her father. The grandparents could be tested, and the variant was *de novo* in the father.

Patient 2 is the first boy of two children born to non-consanguineous parents. Family history was unremarkable. His mother works as a company manager. Following an uncomplicated pregnancy obtained by in vitro fertilization, the proband was born at 38 weeks of gestation. His height was at 20th percentile (p), weight was at 8th p and HC at 5th p. Development was delayed: his first words were after two years old, so he required speech therapy. He walked at 16 months and showed important difficulties in fine motor skills for which he needed psychomotor and occupational therapists. He also showed attention deficit and anxiety disorder, and a learning disability, causing him to repeat the first grade. Brain magnetic resonance imaging (MRI) was normal. At 4 years and 11 months, his height, weight and HC were 110 cm (+1 SD), 17 kg (+1 SD) and 49 cm (-2 SD), respectively. No dysmorphic features were noticed.

Previous testing included a normal chromosomal microarray and *FMR1* methylation analysis. Analysis of a panel of genes involved in intellectual disability identified a pathogenic deletion in *MBD5* c.4042_4051del, p.(Ser1348Glnfs*3), inherited from his mother. The grandparents could be tested, and the variant was *de novo* in the mother.

Patient 3 is the only child born to non-consanguineous parents. His father showed moderate hearing loss requiring hearing aids. He works as an audioprothesist. The proband was born at 38 weeks of gestation with intrauterine growth retardation. His height was 45,5 cm (3rd p), weight was 2760 g (10th p), and HC was 31 cm (<3rd p). Development was delayed: his first words were at 18 months old. He walked at 15 months and showed difficulties in fine motor skills. He also showed an autism spectrum disorder (Autism Diagnostic Observation Schedule at 14 at 19 months) with agitation,

concentration disorder and learning disability. Care and support included a psychomotor and speech therapist, and an occupational therapist and educator. Brain MRI and renal ultrasound were normal. At 4 years and 6 months, his height, weight and HC were 95 cm (-2,5 SD), 13 kg (-2,5 SD) and 47,5 cm (-3 SD), respectively. Dysmorphic features were noted: trigonocephaly, broad nose and large mouth. He also had significant skin atopy.

Previous testing included a normal chromosomal microarray and *FMR1* methylation analysis. ES identified a pathogenic variant in *KMT2C* c.8849_8850del, p.(His2950Argfs*17) inherited from his father. The variant was also identified from DNA extracted from the father's skin biopsy. The grandparents could be tested, and the variant was *de novo* in the father.

Patient 4 was born to non-consanguineous parents. Family history was unremarkable. His mother has a post high-school education. Following an uncomplicated pregnancy, the proband was born at full term, with normal measurements. He showed a congenital cardiopathy (atrio-ventricular septal defect) and a significant velar insufficiency which required surgery. He walked at one year old. He had a learning disability and followed normal schooling with support. At 14 years and 6 months, his height, weight and HC were 171 cm (+1 SD), 52,4 kg (mean) and 54,5 cm (-0,5 SD), respectively. Dysmorphic features were noted: telecanthus, ptosis, short philtrum, protruding nose.

Previous testing included a karyotype, a chromosomal microarray and a panel of genes involved in clefts, and all were normal. ES identified a pathogenic variant in *ZMIZ1* c.253C>T, p.(Arg85*) inherited from his mother. The variant was identified in both mother's blood and saliva samples.

Patient 5 is the second girl of two children born to non-consanguineous parents. Family history was unremarkable other than a learning disability in the father, who is a painter in the building construction field. The pregnancy was unremarkable. Development was delayed: she walked at 18 months old and showed a language delay that required speech therapy. She showed a mild intellectual

disability and needed support at school. She has an astigmatism and a dystonia. Brain MRI revealed a delayed myelination. At 16 years and 8 months, her height, weight and HC were 151 cm (-2 SD), 45 kg (-2 SD) and 55 cm (-0,5 SD), respectively. Dysmorphic features were noted: large eyebrows, broad nose tip, small ears. She also showed short hands and feet with short middle phalanx.

Previous testing included a normal chromosomal microarray. ES identified a pathogenic variant in *KMT2E* c.5226dup, p.(Pro1743Thrfs*126), inherited from her father.

Patient 6 is the first boy of three children born to non-consanguineous healthy parents. His two brothers also showed neurodevelopmental disorders. One maternal aunt showed mild intellectual disability with epilepsy, and one maternal great-uncle showed a severe intellectual disability. His father obtained a Youth Training diploma in market gardening. Following a pregnancy marked by the mother's hypertension, the proband was born at full term, with normal measurements. Development was delayed: his first words were at 3 years old and he walked at 16 months. He required a psychomotor and emotional management therapy. He showed intellectual disability (WISC score 72) and repeated the first grade. He also showed a severe asthma and an astigmatism. Brain MRI revealed a Chiari type 1 malformation (also found in his parents) and an asymptomatic syringomyelia. At 5 years and 8 months, his height, weight and HC were 102 cm (-2,5 SD), 15 kg (-2,5 SD) and 52,5 cm (+0,5 SD), respectively. No dysmorphic features were noted, he just had vitiligo on his forehead.

Previous testing included a normal chromosomal microarray and *FMR1* methylation analysis. Analysis of a genome identified a pathogenic variant in *KMT2E* c.685A>T, p.(Lys229*) inherited from his father. This variant was also identified in his two brothers.

Patient 7 is boy, only child of the couple, born to non-consanguineous parents. Family history was unremarkable. His mother obtained a Youth Training diploma and works as a waitress. Following an

uncomplicated pregnancy, the proband was born at 38 weeks of gestation. His height was 47 cm (13th p), weight was 2970 g (33rd p) and HC was 33,5 cm (28th p). Several congenital malformations were observed: laryngomalacia, cerebral malformations, lateral right semicircular canal hypoplasia. He also showed gastroesophageal reflux disease, oral disorders, severe sleep apnea, strabismus, inguinal hernia, bilateral testicular ectopia and micropenis. He needed growth hormone treatment for a growth failure. Development was delayed: he walked at 4 years old and used bisyllabic speech at 9 years. He showed intellectual disability. Brain MRI described several cerebral malformations: abnormalities of gyration, corpus callosum dysmorphism, Chiari type 1 malformation and partial rhombencephalosynapsis. At 9 years and 4 months, his height and weight were 128,2 cm (-1 SD) and 26,5 kg (-0,5 SD) respectively. His last HC was 50,5 cm at 7 years old (-1 SD). Other clinical features were noted: skin xerosis, kyphoscoliosis and turricephaly without craniosynostosis. Dysmorphic features were also noted : hypertelorism, downslanting palpebral fissures, midface hypoplasia, flat face, brachycephaly, bitemporal narrowing long, upturned nose, high-arched palate, dysplastic, low-set and posteriorly rotated ears, overfolded helix, thin upper lip vermilion.

Previous testing included a normal karyotype, chromosomal microarray, *FMR1* methylation analysis and various targeted analysis and ES. Genome sequencing identified a pathogenic nonsense variant in *MN1* c.3778G>T, p.(Glu1260*) inherited from his mother. The grandparents could be tested, and the variant was *de novo* in the mother. The variant was identified in both mother's blood and saliva samples.

Patient 8 is the second girl of three children born to non-consanguineous parents. Family history was unremarkable other than two miscarriages. Her mother doesn't show any intellectual disability and is a stay-at-home mom. Following an uncomplicated pregnancy, the proband was born at full term, with normal measurements. She showed linear atrophic lesions on the face and neck since birth. Motor development was delayed (she walked at 22 months old) but language development was normal. She

showed intellectual disability, and abnormal behavior with agoraphobia. An ultrasound revealed a uterine malformation. At 16 years and 10 months, her height, weight and HC were 158,7 cm (-1 SD), 55,5 kg (M) and 49,5 cm (-4 SD), respectively. No dysmorphic features were noted but she showed skin linear atrophic lesions.

ES identified a pathogenic variant in *NDUFB11* c.145_152dup, p.(Thr52Glnfs*66), inherited from her mother. The grandparents couldn't be tested and the proband's healthy sister does not carry the variant.

Patient 9 is the last of three children born to non-consanguineous parents. Family history was unremarkable. His father has a post axial longitudinal hemimelia with facial asymmetry. He obtained a Business Technician Education Council First Diploma and a Youth Training. Following an uncomplicated pregnancy, the proband was born at full term, with normal measurements. Development was delayed: he walked at 18 months and showed coordination disorder. Around 18 months, he began flapping stereotypies and had less eye contact, leading to the diagnosis of severe autism spectrum disorder. At 9 years old he spoke using bisyllabic words. At 9 years, his height, weight and HC were 134 cm (+1 SD), 32 kg (+1,5 SD) and 55 cm (+1 SD), respectively. Dysmorphic features were noted: hypertelorism and wide mouth.

Previous testing included a normal chromosomal microarray and *FMR1* methylation analysis. ES identified a pathogenic deletion in *CUL3* c.883+3_883+6del, inherited from his father.

Patient 10 is the only girl born to non-consanguineous parents. Family history was unremarkable. Her mother had mild learning disability and obtained a Youth training in sewing. Ultrasound during pregnancy revealed hydramnios, vertebral malformations, right renal agenesis and suspected esophageal atresia. The proband was born at full term, with a weight at 2780g (70^{th} p). The polymalformative syndrome was confirmed at birth: cardiopathy (anomalous pulmonary venous

return, atrial septal defect and patent ductus arteriosus), right renal agenesis, vertebral segmentation defect, posterior cleft palate, right ear malformation (agenesis of the auricle, atretic ear channel, rock malformation, pre-auricular fibrochondroma). During neonatal period, she had oral disorders and required nasogastric feeding and oxygen therapy during her first year. She had an arthrodesis for a severe scoliosis. Development was delayed: she walked at two years old and pronounced only five words at 12 years old. She showed abnormal behavior with stereotypy, agitation, aggression. At 13 years and 4 months, her height and weight were 144 cm (-2 SD) and 44,5 kg (M) respectively. Dysmorphic features were noted: micrognathia and right mandibular hypoplasia. She also showed a bilateral hallux valgus, and partial syndactyly of the 2nd and 3rd toes.

Previous testing included a normal chromosomal microarray and *FMR1* methylation analysis. ES identified a pathogenic variant in *MED13* c.4368C>A, p.(Cys1456*) inherited from her mother. The grandparents couldn't be tested but the variant was found in a maternal aunt.

Patient 11 is the only girl of two children born to non-consanguineous parents. Family history was unremarkable other than a chronic lymphocytic leukemia in the mother. Her father is a national Gendarmerie officer. Following an uncomplicated pregnancy, the proband was born at 38 weeks of gestation with intrauterine growth retardation. Her height was 42,5 cm (<1st p), weight was 2200 g (1st p) and HC was 32,5 cm (18th p). At birth, she showed congenital hip dysplasia which required surgery, oral disorders with a surgically treated gastroesophageal reflux disease and vesicoureteral reflux. She had recurrent infections in childhood. Skeletal examination showed a scoliosis. Development was delayed: she walked at two years old, and her first words were pronounced after three years old. She showed a mild intellectual disability and needed support at school. She had sleep disorder. Brain MRI was normal. At 12 years, her height and weight were 151 cm (M) and 38 kg (+1 SD) respectively. Dysmorphic features were noted: wide nose with bulbous tip, sparse eyebrows and down turning corners of the mouth.

ES identified a pathogenic deletion in *ARID2* c.-2_284+2del, inherited from her healthy father.

Patient 12 is the only child born to non-consanguineous parents. Family history was unremarkable other than a breast cancer in his mother and a kidney cancer in his father. His mother has a Youth Training as a saleswoman. Following an uncomplicated pregnancy, the proband was born at full term, with normal measurements. He walked at 17 months. Language was delayed: his first words were at two years and six months old. He also had attention deficit and was diagnosed with autism. He showed a moderate intellectual disability and needed support at school. Care support included psychomotor and speech therapy, as well as psychological care. Brain MRI was normal. At 11 years and 2 months, his height, weight and HC were 138 cm (-1 SD), 36 kg (+1 SD) and 53 cm (-0,7 SD), respectively. No dysmorphic features were appreciated.

Previous testing included a normal chromosomal microarray, *FMR1* methylation analysis and research for Angelman syndrome. Exome sequencing identified a pathogenic duplication in *RERE* c.3249dup, p.(Ser1084Valfs*19) inherited from his mother. The grandparents could be tested, and the variant was *de novo* in the mother.

Table 1. Detailed clinical features of the patients

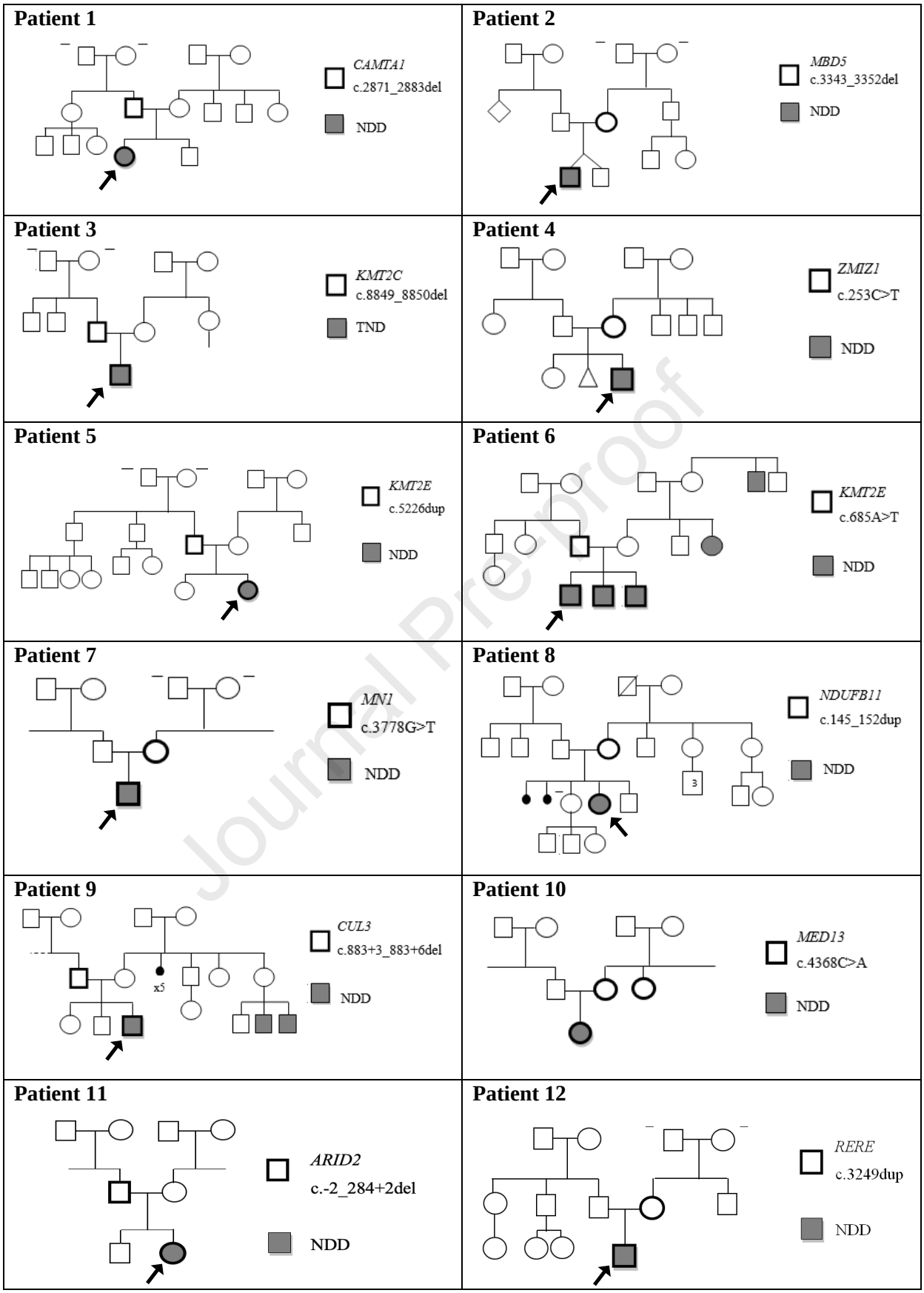
Patient	**Patient 1**	**Patient 2**	**Patient 3**
Gene	*CAMTA1*	*MBD5*	*KMT2C*
pLI (gnomAD v2.1)	1	1	1
observed/expected LoF ratio (gnomAD v2.1)(o/e and 90% CI)	0.09 (0.05 - 0.17)	0.04 (0.02 - 0.14)	0.08 (0.06 - 0.12)
de novo LoF in controls (denovodb) *accessed september 22, 2023*	No	No	No
Variation*	NM_015215.4 Chr1(GRCh37): g.7737750_7737762del c.2871_2883del p.(Thr959Profs*31)	NM_001378120.1 Chr2(GRCh37): g.149247243_149247252del c.4042_4051del p.(Ser1348Glnfs*3)	NM_170606.3 Chr7(GRCh37): g.151873688_151873689del c.8849_8850del p.(His2950Argfs*17)
Method of identification	Exome sequencing	Panel	Exome sequencing
ACMG criteria	PVS1, PP5, PM2	PVS1, PM2	PVS1, PP5, PM2
Interpretation	Likely pathogenic	Likely pathogenic	Likely pathogenic
ClinVar Accession number	VCV000916056.2	ND	VCV000978402.1
additional ClinVar submissions	no	no	no
GnomAD (v2, v3 and v4- Passed RF filter)	Absent	Absent	1 in gnomAD v4, exomes, probable mosaic
DDD *accessed september 22, 2023*	Absent	Absent	Patient 281016 - Heterozygous, *de novo* Pathogenic - Full contribution
Denovo db (variant in patients) *accessed september 22, 2023*	Absent	Absent	Absent

Inheritance	father	mother	father
Transmitting parent : epilepsy ?	No	No	No
Transmitting parent : dysmorphy ?	No	No	No
Transmitting parent : special education ?	No	No	No
Grandparents tested	Yes : *de novo*	Yes : *de novo*	Yes : *de novo*
Index individual			
Gender	Female	Male	Male
Age at assessment	5 years 6 months	4 years 11 months	13 months
Neurological abnormalities (index individual)			
Intellectual disability	+	+	+
Motor developmental delay	+	-	-
Speech delay	+	+	+
Abnormal behavior	Autism spectrum disorder	Anxiety, attention disorder	Autism spectrum disorder
Brain MRI	-	Normal	Normal
Other clinical features (index individual)			
Age at last assessment	9 years 11 months	4 years 11 months	4 years 6 months
Measurements	H: 135cm (M) W: 26,7kg (-0,5 SD) HC: 51cm (-1 SD)	H: 110cm (+1 SD) W: 17kg (+1 SD) HC: 49cm (-2 SD)	H: 95cm (-2,5 SD) W: 13kg (-2,5 SD) HC: 47,5cm (-3 SD)

Craniofacial features	-	-	trigonocephaly, broad nose and wide mouth
Clinical features	hypertrichosis of the elbow velar insufficiency	-	skin atopy
Congenital malformations	-	-	-
Other findings			
		IUGR	IUGR

** Nomenclature HGVS V2.0 according to mRNA reference sequence. Nucleotide numbering uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.*

Abbreviations : pLI : probability of Loss of function Intolerance; SD: standard deviation; H: Height ; W : Weight ; HC : Head Circonference ; AVSD : Atrioventricular septal defect ; APVR : Anomalous pulmonary venous return ; ASD : Atrial septal defect ; CC : Corpus Callosum ; CT1 : Chiari Type 1 ; GERD : Gastroesophageal reflux disease ; IUGR : Intrauterine growth retardation ; PDA : Patent ductus arteriosus, ND: not documented/no data



Abbreviation : NDD : Neurodevelopmental Disorder
-: tested negative for the variation

Figure 1: Pedigree of the 12 families