Variants in MED12L, encoding a subunit of the Mediator kinase module, are responsible for intellectual disability associated with transcriptional defect

Mathilde Nizon, MD1,11, Vincent Laugel, MD, PhD2, Kevin M. Flanigan, MD3, Matthew Pastore, MS, LGC3, Megan A. Waldrop, MD3, Jill A. Rosenfeld, MS4, Ronit Marom, MD, PhD4, Rui Xiao, PhD4, Amanda Gerard, MS, CGC4, Olivier Pichon, MS1, Cédric Le Caignec, MD, PhD1, Marion Gérard, MD5, Klaus Dieterich, MD6, Megan Truitt Cho, MS, CGC7, Kirsty McWalter, MS, CGC7, Susan Hiatt, MD8, Michelle L. Thompson, MD8, Stéphane Bézieau, PharmD, PhD10,11, Alexandra Wadley, MD9, Klaas J. Wierenga, MD10, Jean-Marc Egly, PhD11, Bertrand Isidor, MD, PhD1,12

1CHU Nantes, Service de Génétique Médicale, Nantes, France
2Laboratoire de génétique médicale, INSERM UMR 1112, Faculté de Médecine, 11 rue Humann, 67000 Strasbourg, France
3Neurology Department, Nationwide Children’s Hospital, Columbus, Ohio, USA
4Baylor College of Medicine, Houston, Texas, USA
5Service de Génétique Clinique, CHU Caen, Caen, France
6Service de Génétique Clinique, CHU Grenoble, Grenoble, France
7Clinical Genomics GeneDx, Gaithersburg, Maryland, USA
8HudsonAlpha Institute for Biotechnology, Huntsville, Alabama, USA
9University of Oklahoma School of Medicine, Oklahoma City, OK, USA
10Mayo Clinic Florida, Jacksonville, FL 32224, KW, USA
11Institut de génétique et de biologie moléculaire cellulaire IGBMC, Faculté de Médecine, Strasbourg, France

CONFLICT OF INTEREST STATEMENT
Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics. Megan Truitt Cho and Kirsty McWalter are employees of GeneDx, Inc., a wholly owned subsidiary of OPKO Health, Inc.

The other authors declare no conflict of interest.

ACCESSION NUMBERS
The accession numbers for the SNPs and CNVs reported in this paper are DECIPHER: 284908, 280845 and 277489; and ClinVar: SCV000611598, SCV000853261, SCV000853262 and SCV000853263.

SUPPLEMENTAL DATA
Supplemental data includes one text file with detailed clinical data.
Abstract

**Purpose**—Mediator is a multiprotein complex that allows the transfer of genetic information from DNA binding proteins to the RNA polymerase II during transcription initiation. MED12L is a subunit of the kinase module, which is one of the four sub-complexes of the mediator complex. Other subunits of the kinase module have been already implicated in intellectual disability, namely MED12, MED13L, MED13 and CDK19.

**Methods**—We describe an international cohort of seven affected individuals harboring variants involving MED12L identified by array CGH, exome or genome sequencing.

**Results**—All affected individuals presented with intellectual disability and/or developmental delay, including speech impairment. Other features included autism spectrum disorder, aggressive behavior, corpus callosum abnormality and mild facial morphological features. Three individuals had a MED12L deletion or duplication. The other four individuals harbored single nucleotide variants (one nonsense, one frameshift and two splicing variants). Functional analysis confirmed a moderate and significant alteration of RNA synthesis in two individuals.

**Conclusion**—Overall data suggest that MED12L haploinsufficiency is responsible for intellectual disability and transcriptional defect. Our findings confirm that the integrity of this kinase module is a critical factor for neurological development.

**Keywords**

MED12L; intellectual disability; mediator complex; transcriptional defect; corpus callosum

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**INTRODUCTION**

Mediator is a key regulator of gene expression involved in cell growth, homeostasis, development and differentiation. Mediator is a large multiprotein complex composed of four different modules (Kinase, Head, Middle and Tail), which conveys essential information from proteins bound at DNA response elements to the basal transcription machinery located around the transcription initiation site. Dysfunction of the transcription machinery components, including Mediator, has been shown to elicit a range of effects on cell states giving rise to diverse disorders including developmental delay and intellectual disability. Variants in Mediator subunits are associated with a wide range of genetic disorders, most of them exhibiting neurological disabilities. Genetic and biochemical studies have established the Mediator Kinase module as a major ingress of developmental and oncogenic signaling through the three other modules constituting the core component, and much of its function derives from its resident CDK8 kinase activity likely regulated by its association with MED13, MED12 and Cyclin C (CycC) subunits. Recent studies have shown that the kinase module can also encompass CDK19, MED12L, and MED13L, which are paralogs of CDK8, MED12, and MED13 respectively. Little is known about their biological roles, but each of these proteins appears to assemble in a mutually exclusive fashion with its paralog.
Germline variants of *MED12* have already been found in several genetic disorders associated with X-linked intellectual disability, such as Opitz-Kaveggia syndrome also named FG syndrome\(^6\)–\(^8\), Lujan syndrome (p.N1007S)\(^9\) and Ohdo syndrome\(^10,11\). All of these MED12-related disorders exhibit defects in gene expression\(^12\). Variants in CDK19, *MED13* and *MED13L* have also been associated with neurodevelopmental disorders\(^13\)–\(^15\), likely due to defects in gene transcription.

Here, we report a series of individuals sharing variants involving *MED12L* (mediator complex subunit 12 like) [OMIM 611318] recruited through an international collaboration and identified by array Comparative Genomic Hybridization (aCGH), Exome or Genome Sequencing. Our attention focused on the effect of *MED12L* on gene expression, studying transcription machinery of two individuals’ fibroblasts. Whereas CDK19, MED12L, and MED13L are now all linked to neuronal and developmental disorders, their basic biological importance relative to CDK8, MED12, or MED13 remains unclear.

**MATERIALS AND METHODS**

**Individual recruitment**

The compilation of this case series resulted from an international collaborative effort among Centre Hospitalier Universitaire (CHU) Nantes, Strasbourg University, CHU Caen, Baylor Genetics Laboratories (BG), GeneDX, HudsonAlpha Institute for Biotechnology and University of Oklahoma School of Medicine. It was also partly facilitated by the web-based tools GeneMatcher and DECIPHER\(^16,17\). All participants were clinically assessed by at least one clinical geneticist from one of the participating centers. The study was approved by the CHU de Nantes-ethics committee (number CCTIRS: 14.556). Consents for the publication of photographs (Figure 1) were obtained for individuals 1, 2 and 5.

**Molecular analysis**

Institutional review board-approved written informed consents were obtained for all subjects. DNA was extracted from leukocytes according to standard procedures.

Copy number variants (CNV) were found by microarray-based comparative genomic hybridization (array CGH). Different array platforms were used for genomic copy number analyses which were carried out according to manufacturers’ recommendations: Agilent CGH Microarray 60K or 180K (Agilent Technologies, Santa Clara, CA). Chromosomal rearrangements were confirmed by fluorescence in situ hybridization (FISH) with various specific probes on chromosome preparations from leukocyte cultures or by quantitative polymerase chain reaction (PCR) using standard protocols. Several different oligonucleotide probes were used to try to sequence the breakpoints of the CNV carried by individual 1. Parental testing was performed when DNA samples were available. Genomic positions are relative to human genome Build GRCh37/hg19. The CNVs were submitted to the DECIPHER database.

Single nucleotide variants (SNV) were all identified by exome or genome sequencing. The protocols used by each participating center have been detailed elsewhere\(^18\)–\(^19\). Reads were aligned to the human reference genome sequence (NCBI build 37.3, hg19). For SNV,
segregation analysis was made by direct sequencing and then performed in the families to confirm inheritance when parental DNA samples were available. The variants were submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

RRS assay

Recovery of RNA synthesis (RRS) was evaluated on primary fibroblast cultures using fluorescent non-radioactive assay as described previously. RRS evaluates the transcription-coupled repair pathway (TC-NER). Cells were plated on coverslips in 6-well plates at a confluence of 7x10^4 cells per well. After 2 days in culture, cells were washed with phosphate-buffered saline (PBS), followed by irradiation with a range of UV-C doses (6–12-20 J/m^2). The non-irradiated plates acted as references. After UV-irradiation, cells were incubated for 23 h for RNA Synthesis recovery in DMEM (Dulbecco’s modified Eagle’s medium) supplemented with fetal bovine serum. Then, after washing with PBS, cells were labeled with 5-ethylidene-uridine (EU; Invitrogen) for 2 h. Cells were then washed again with PBS, followed by fixation and permeabilization. The last step involved an azide-coupling reaction and DAPI (6'-diamino-2-phenylindole) staining (Click-iT RNA HCS Assay, Invitrogen). Finally coverslips were washed in PBS and mounted on glass slides with Ibidi Mounting Medium (Biovalley). Photographs of the cells were taken with a fluorescent microscope (Imager.Z2) equipped with a CCD (charge-coupled device) camera (AxioCam, Zeiss). The images were processed and analyzed with the ImageJ software. At least 50 cells were randomly selected, and the average nuclear fluorescent intensity was calculated.

RESULTS

Clinical description

We identified seven affected individuals from seven independent families, including four males and three females. The main clinical features of our cohort are summarized in Table 1 and Figure 1 and detailed in Supplementary data.

All individuals had neurological impairment. Intellectual disability/developmental delay was the main feature (7/7). Three individuals had a mild intellectual disability, three had a moderate intellectual disability with mild to severe speech impairment. One individual had a severe intellectual disability with seizures and brain abnormalities. Abnormal behavior was common (6/7) including mild to severe autism spectrum disorder (4/7) and aggressive behavior (4/7). Attention deficit with hyperactivity (3/7), sleep disorder (3/7) and hypotonia (1/7) were also noted. One individual had seizures and another one had staring spells with the EEG within normal limits. Brain MRI was performed in four individuals showing abnormalities for three of them: corpus callosum agenesis or hypoplasia (2/4), and secondary cortical signal abnormality with volume loss of bilateral putamen and globus pallidus (1/4).

Gastrointestinal issues were reported in 5/7 individuals, including chronic constipation (3/7), feeding difficulties (2/7, one individual required a gastrostomy tube placement), gastroesophageal reflux (2/7) and inguinal hernia (1/7). Various other miscellaneous extra-neurological features were reported such as congenital malformations (2/7, including iris...
coloboma and hypospadias), ophthalmologic features (3/7), skeletal abnormalities (2/7) and minor hand anomalies (4/7). Mild facial morphological features were reported in some individuals, but without a recognizable phenotype.

**Genetic results**

Genomic positions of copy number variants (CNVs) and single nucleotide variants (SNVs) identified in MED12L are mapped in Figure 2.

Individuals 1, 2 and 3 harbored CNVs involving MED12L ranging in size from 147 Kb to 460 Kb (DECIPHER accession number: 284908, 280845 and 277489). Individual 1 carried a 460 Kb duplication involving the terminal part of MED12L. Attempts to sequence the breakpoints of the duplication by Sanger failed arguing for a complex rearrangement. Individual 2 carried a 291 Kb deletion involving the terminal part of MED12L. Individual 3 carried a 147 Kb intragenic MED12L duplication. Minimal and maximal coordinates of the CNVs are indicated in Table 1. CNVs were found to be de novo in individuals 1 and 2. Parents were not available for individual 3.

Genome sequencing was performed in individual 4 and exome sequencing in individuals 5 to 7. One frameshift, one nonsense and two splice variants were identified in MED12L (NM_053002.5, exons are numbered like in NG_021244.1): c.1747dup, p. (Ser583PhefsTer8), c.5992C>T, p.(Gln1998Ter), c.4374–1G>A, c.4686–1G>A (ClinVar accessions: SCV000611598, SCV000853261, SCV000853262 and SCV000853263). Variants were absent from GnomAD, EVS and 1000 Genome databases. Nonsense variants likely result in the degradation of mRNA by nonsense-mediated decay. Segregation analysis showed that the variants occurred de novo when parents’ DNA was available (individuals 5 and 7).

Lastly, individual 4 harbored a variant of unknown significance in TUBB2B [OMIM 612850] that could not be tested for inheritance or excluded as contributing to the individual overall phenotype (see table 1).

**Functional analysis**

To test the functional consequences of the MED12L variants on transcriptional processes, we used the recovery of RNA synthesis (RRS) assay, which is the gold standard diagnostic tool in Cockayne syndrome (CS), another transcription-related human disease with syndromic intellectual disability [21]. This assay reflects the global transcriptional activity by measuring the incorporation of fluorescent uridine 24 hours after UV irradiation. In wild type cells, UV irradiation temporarily halts the transcription of a large subset of genes, and 24 hours later they recover a normal RNA synthesis activity [22]. Such an assay is used to partly synchronize cells and enable a standardized assessment of global transcriptional activity. Primary fibroblasts from two individuals with MED12L CNV (individuals 1 and 2) were available for RRS testing and were compared to fibroblasts from a healthy control, from a classical Cockayne individual (carrying pathogenic variants in the ERCC8/CSA gene) and from individuals carrying pathogenic variants in other mediator subunit genes, MED12 and MED13L. Both MED12L cell lines show a moderate but significant decreased RNA synthesis level 24 hours after UV irradiation, as compared to the healthy control.
similar to the level observed in a control MED12 cell line (Figure 3). The control MED13L cell line showed a more severely reduced RNA synthesis level, closer to the classical and the severe transcriptional defect observed in the control Cockayne cell line.

**DISCUSSION**

Here we described a series of seven individuals presenting with variants (CNVs and SNVs) involving MED12L. Individual 1 carried a partial de novo duplication of MED12L. Despite not being proven by our study, we suspected a complex rearrangement. We also showed that the duplication was associated with a defect in transcriptional activity of fibroblasts. Individual 2 carried a partial deletion with a similar transcriptional defect as for individual 1. Individuals 3, 4 and 5 carried respectively an intragenic duplication, a nonsense variant (localized in exon 39/43), and a frameshift variant with a premature nonsense variant, all predicted to result in an altered mRNA likely eliminated by nonsense-mediated decay (NMD). Individuals 6 and 7 carried intronic variants predicted to alter splicing resulting in a premature nonsense codon. Most variants were de novo. Unfortunately, segregation was not possible for three patients either because they were adopted (individuals 4 and 6) or because parents’ DNA were not available (individual 3). Functional studies were performed only for two French patients. Fibroblasts were not available for the other individuals. All in all, despite these limitations, those data argue for a haploinsufficiency mechanism leading to transcriptional defect.

All individuals described here showed intellectual disability/developmental delay or speech impairment, sometimes associated with abnormal behavior, corpus callosum agenesis, and mild facial morphological features. No genotype-phenotype correlation could be identified in our series. To note that individual 1 also harbored a 22q11.2 duplication, which is considered as a risk factor for attention deficit hyperactivity disorder, autism and intellectual disability with a low penetrance estimated around 10%.

MED12L contains 43 exons and encodes a protein component of Mediator, which is involved in transcriptional coactivation of nearly all RNA polymerase II-dependent genes. MED12L is localized to the nucleus and mainly expressed in brain. By homology, three domains are predicted to be common with MED12L, including a LCEWAV-domain (AA 283–730), with a conserved sequence motif of unknown function, and a catenin-binding domain (AA 1802–2019), activating the canonical Wnt/beta-catenin pathway. The measure of probability of intolerance to loss of function (pLI) score, based on the difference in observed and expected loss-of-function variants in the gene, indicates MED12L is extremely intolerant to heterozygous loss of function (pLI score of 1). MED12L overlaps with six genes, five of which P2RY12, P2RY13, P2RY14, GPR171 and GPR87 encode purinergic receptors participating in vascular and immune response to injury, and currently have no known link to neurodevelopment. MED12L also overlaps IGSF10 which encodes an immunoglobin involved in the control of early migration of neurons expressing gonadotropin-releasing hormone. A MED12L variant (rs1554120) was associated
(p = 5.25E-06) with cortical thickness of right Heschl’s gyrus (HG) by genome-wide association study \(^{25}\). HG is a core region of the auditory cortex whose morphology is highly variable across individuals. This variability has been linked to sound perception ability in both speech and music domains. MED12L interacts with NCAPD2 in human \(^{26}\). NCAPD2 encodes a non-SMC (Structural maintenance of chromosomes) subunit of the condensin complex and causes autosomal recessive microcephaly \(^{27}\).

Mediator is highly conserved across eukaryotes \(^{22}\). It links gene-specific transcription activators with the basal transcription machinery. Mediator contains 30 subunits organized into four modules: MED12L is predicted to be a subunit of the kinase module with MED12, MED13, MED13L, CDK8, CDK19 and Cyclin C. The kinase module is reversibly associated with the Mediator core and is involved in transcriptional repression and activation. The function of this module could be mediated by its kinase activity when it is recruited to an upstream activation region or enhancers, or by the mutually exclusive interaction between the kinase module and RNA polymerase II with the Mediator core integrating the pre-initiation complex \(^{28,29}\). The Mediator complex is also involved in chromatin regulation, and mRNA processing \(^{3,30,31}\).

Other Mediator kinase module subunits have already been implicated in human disease, namely MED12, MED13, MED13L and CDK19. Variants in MED12 have been reported in Opitz-Kaveggia (FG) syndrome, Lujan-Fryns syndrome and Ohdo syndrome, Maat-Kievit-Brunner type \(^{6,8–10}\). Those three X-linked intellectual disability syndromes are allelic and caused by missense MED12 variants and defects in gene expression. FG syndrome and Lujan-Fryns syndrome share some overlapping features such as dysgenesis of the corpus callosum, relative macrocephaly, a tall forehead and hypotonia. Chronic constipation or anal anomalies are a frequent finding in individuals with FG syndrome. Males with FG syndrome also have deficits in communication skills despite affability and excessive talkativeness, attention deficit hyperactivity disorder, maladaptive behavior with aggressive behavior and anxiety \(^{32}\). Marfanoid habitus and hypernasal speech are related to Lujan-Fryns syndrome. Hyperactivity, aggressive behavior, shyness, and attention-seeking behavior are also common in individuals with Lujan-Fryns syndrome. Consistent facial morphological features include downslanted palpebral fissures, high narrow palate, dental crowding and microretrognathia. Ohdo syndrome is responsible for intellectual disability with hyperactivity and aggressive behavior, blepharophimosis, microretrognathia, constipation and feeding difficulties \(^{30}\).

Recently, 13 individuals were described harboring de novo missense and nonsense MED13 [OMIM 603808] variants. All individuals had mild to moderate intellectual disability and speech disorder. Other features mainly included autism spectrum disorder, attention deficit hyperactivity, optic nerve abnormalities, Duane anomaly, hypotonia, mild congenital heart malformations, and dysmorphism \(^{15}\). Missense and nonsense MED13L [OMIM 608771] variants are also responsible for moderate to severe intellectual disability and severe speech disorder with poor language. More than 60 individuals have been reported so far. Other features included hypotonia, autism and behavioral disorder, corpus callosum hypoplasia or agenesis, and miscellaneous malformations \(^{14,33–35}\). Finally, disruption of CDK19 has been
reported in an individual with intellectual disability, microcephaly and bilateral congenital retinal folds. Therefore, all the subunits of the kinase module seem to be crucial for neurological development. Some features are common between the four conditions linked to MED12, MED13, MED12L and MED13L such as intellectual disability, behavior abnormalities and autism spectrum disorders. A CDK19 variant has been reported in only one individual with a different neurological phenotype. Table 2 summarizes common features between the four main MED-related conditions. Corpus callosum anomalies seem common with MED12 and MED12L variants and can be seen less frequently with MED13L variants. MED12 and MED13L variants appear to lead to a more severe condition, with poor speech and facial hypotonia, than MED13 and MED12L related disorders. Finally, the wide range of other organs affected is an inconsistent feature and can be related to the ubiquitous expression of those genes and their broad regulatory role. Mild facial morphological features are common for all those disorders, but does not help in pointing to a clinical diagnosis.

Herein, we demonstrate that MED12L fibroblasts for individual 1 and 2 show a defect in transcriptional activity as measured by recovery of RNA synthesis after UV irradiation. This defect is similar to the defect observed in a MED12 cell line and milder than the defect seen in a MED13L cell line derived from an individual who showed a more severe neurological phenotype. It can be hypothesized that the pathogenic variants in Mediator kinase module subunits could impair the transcriptional co-activation functions of the complex under specific conditions, during development and during postnatal life, leading to the phenotype observed in the individuals. It has already been similarly postulated that neurodevelopmental defects in individuals affected with CS and trichothiodystrophy could be due to transcriptional defects mediated by variants in the other transcriptional coactivation complexes TFIIH or TFIIE.

In conclusion, we report here the involvement of MED12L in human disease as has been seen for other subunits of the kinase module of the mediator complex, through transcriptional defect. Even if many intellectual disability syndromes are challenging to differentiate based on clinical phenotype alone, a common clinical pattern seems to emerge for the disorders related to transcriptional defect linked to Mediator complex.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**AKNOWLEDGEMENTS**

We thank the patients and their families for participating in this study.

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Figure 1: Photographs and brain MRI of individuals with variants in MED12L.
A, Individual 1: unilateral ptosis with iris coloboma, hypertelorism, sparse eyebrows, downslanted palpebral fissures, bulbous nasal tip. B, Individual 2: prominent nasal bridge, short philtrum, everted lower lip, small mouth. C, Individual 5: deep-set eyes, bulbous nasal tip, thin upper lip, triangular face. Brain MRI shows mildly hypoplastic corpus callosum, which is foreshortened with a small splenium.
Figure 2:
A. Genomic position of MED12L variants identified in our series. Upper panel represents single nucleotide variants. Lower panel represents copy-number variants. B. Localization of predicted domains of MED12L protein.
Figure 3: Recovery of RNA synthesis (RRS) following UV irradiation.
Fibroblasts of individuals 1 (Δ) and 2 (○) show a moderately decreased level of RRS as compared to the normal control cell line (♦), similar to a MED12 mutated cell line (●). A MED13L mutated cell line (▯) shows a more severely decreased RRS level, closer to the typical severely decreased level of RRS in a CSA defective cell line (■). Error bars represent standard errors of the mean.
Table 1.
Detailed clinical features of the individuals with nucleotide and copy number variants involving *MED12L*.

NA: not available, SD: standard deviation, OFC: occipital frontal circumference.

| Individual  | Individual 1 (Decipher 284008) | Individual 2 (Decipher 280845) | Individual 3 (Decipher 277489) | Individual 4 SCV000611598 | Individual 5 SCV000853261 | Individual 6 SCV000853262 | Individual 7 SCV000853263 |
|-------------|--------------------------------|--------------------------------|--------------------------------|------------------------------|----------------------------|----------------------------|----------------------------|
| Mutation in *MED12L* (according to NCBI reference sequence NM_053002.5, NC_000003.11) | g.151129252C>T, c.5992C>T, p.(Gln1998Ter) | g.150906260dup, c.1747dup, p.(Ser583PhefsTer8) | g.151097900G>A, c.4374–1G>A | g.151097900G>A, c.4374–1G>A | g.151101870G>A, c.4686–1G>A | g.151101870G>A, c.4686–1G>A |
| Size of CNV (Mb) | 460 Kb duplication | 291 Kb deletion | 147 Kb duplication | | | | |
| Proximal breakpoint (Hg19) | 150,983,389 | 150,876,508 | 150,966,686 | | | | |
| Distal breakpoint (Hg19) | 151,441,372 | 151,167,962 | 151,114,133 | | | | |
| Inheritance | de novo | de novo | NA | NA | de novo | NA | de novo |
| Origin | France | France | France | Ukraine | USA | USA | Caucasian |
| Gender | Male | Male | Male | Female | Male | Female | Female |
| Birth term (WG) | At term | At term | 39 | NA | 37 | NA | 32 |
| Pregnancy complications | − | Acute foetal distress at birth | − | NA | Suspected cardiac anomaly, maternal pre-eclampsia | prenatal drug exposures (cocaine, tobacco) | − |
| Birth weight (grams/SD) | 3160 (−1 SD) | 4000 (+1.5 SD) | 3630 (+1 SD) | NA | 3000 (0 SD) | 1729 (0 SD) | 2404 (−2 SD) |
| Birth length (cm/SD) | 47.5 (−2 SD) | 53 (+1.5 SD) | 50 (0 SD) | NA | 48.5 (0 SD) | 38.1 (−1.5 SD) | NA |
| OFC at birth (cm/SD) | 37 (+1.5 SD) | 35 (+1 SD) | 34 (−0.5 SD) | NA | 34 (+0.5 SD) | NA | NA |
| Age at assessment | 12 years | 22 years | 13 years 8 months | 11 years | 5 years | 8 years | 3 years 10 months |
| Weight (kg/SD) | 27 (−2 SD) | 98 (+6 SD) under neuroleptic | 47 (0 DS) | 34 (−1 SD) | 21.1 (+0.5 SD) | 22.6 (0 SD) | 13.3 (−1 SD) |
| Individual | Individual 1 (Decipher 284008) | Individual 2 (Decipher 280845) | Individual 3 (Decipher 277489) | Individual 4 SCV000611598 | Individual 5 SCV000853261 | Individual 6 SCV000853262 | Individual 7 SCV000853263 |
|------------|--------------------------------|--------------------------------|--------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Height (cm/SD) | 131.5 (−2.5 SD) | 185 (+2 SD) | 156 (0 DS) | 142 (−0.5 SD) | 109 (−0.5 SD) | 122.5 (0 SD) | 91.5 (−2 SD) |
| OFC (cm/SD) | 53 (−1 SD) | 57 (+1 SD) | 56 (+1.5 SD) | NA | 50.1 (−0.5 SD) | 49.5 (−1.5 SD) | NA |
| Neurological abnormalities | | | | | | | |
| Intellectual disability | moderate | moderate | mild (IQ 74) | moderate | mild | mild | severe |
| Hypotonia | − | − | − | NA | + | − | + |
| Motor delay | + (walking at 19 months) | − (walking at 16 months) | − | NA | + (walking at 18 months) | + (walking at 20 months) | + |
| Speech impairment | + (mild, sentences at 4 yo) | + (severe, can associate words) | + (pronunciation) can make a conversation | + (speech delay) | + (pronunciation), but good vocabulary and can make a conversation | + (speech delay) | + (no language) |
| Abnormal behavior | + | ++ | + | − | + | + | + |
| Aggressive behavior | − | + | ++ | − | + | ++ | − |
| Autistic features | + | ++ | + | − | + | − | − |
| Anxiety | ++ | + | − | − | − | − | − |
| Attention deficit | − | + | − | − | + | + | − |
| Hyperactivity | − | − | − | − | + | + | − |
| Sleeping disorder | − | + | − | − | + | − | + |
| Seizures | − | − | − | − | − | staring spells | + |
| Abnormal EEG | NA | − | NA | − | − | − | + |
| Abnormal brain magnetic resonance imaging | NA | NA | NA | Agenesis of the Corpus callosum, enlargement of the posterior aspect of the right and left lateral ventricle | Mildly hypoplastic corpus callosum | Normal | Cortical signal abnormality and volume loss of bilateral putamen and globus pallidus at 3 years |
| Extra-neurological abnormalities | | | | | | | |
| Individual | Individual 1 (Decipher 284908) | Individual 2 (Decipher 280845) | Individual 3 (Decipher 277489) | Individual 4 SCV000611598 | Individual 5 SCV000853261 | Individual 6 SCV000853262 | Individual 7 SCV000853263 |
|------------|-------------------------------|-------------------------------|-------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Gastrointestinal anomalies | Chronic constipation, neonatal occlusive syndrome, encopresia | Gastroesophageal reflux | unilateral inguinoscrotal hernia | − | Feeding difficulties in early infancy, moderate chronic constipation | − | Feeding difficulties (G-tube dependent), gastroesophageal reflux, intermittent constipation |
| Congenital malformations | Unilateral coloboma of iris and retina | − | − | − | Suspected VSD prenatally but normal echocardiogram at birth, Hypospadias, voiding dysfunction | − | − | − |
| Skeletal abnormalities | Thoracolumbar kyphosis, hyperlaxity | − | − | Very large knees, appears to have bony prominence medially | − | − | − |
| Hands and feet anomalies | Long appearing fingers, unilateral single palmar transverse crease | Long appearing fingers | Bilateral 5th finger brachyphalangy P1, pes planus | Fingers-fetal padding, 5th hypoplastic nails | − | − | − |
| Sensory abnormalities | Hypermetropia | − | Myopia | − | − | − | − | − | Hypermetropia, strabismus |
| Other findings | − | Dilated cardiomyopathy (toxic origin) | − | Hypopigmented macules (oval shaped on right shoulder blade) | recurrent respiratory infections | − | − | − |
| Dysmorphic features | High forehead | − | − | − | − | − | − | − |
|−| − | − | − | − | − | − | − | − |
|−| − | − | − | − | − | − | − | − |
|−| − | − | − | − | − | − | − | − |
|−| − | − | − | − | − | − | − | − |
|−| − | − | − | − | − | − | − | − |
|−| − | − | − | − | − | − | − | − |
| Individual | Individual 1 (Decipher 284908) | Individual 2 (Decipher 280845) | Individual 3 (Decipher 277489) | Individual 4 SCV000611598 | Individual 5 SCV000853261 | Individual 6 SCV000853262 | Individual 7 SCV000853263 |
|------------|--------------------------------|--------------------------------|--------------------------------|---------------------------|---------------------------|---------------------------|--------------------------|
| High, narrow palate | − | − | − | + | − | − | + |
| Other | Unilateral ptosis, hypertelorism, sparse eyebrows | Short philtrum, everted lower lip, small mouth | − | medial eyebrow flare, inverted lower eyelid, pointed chin, high cheek bones, downturned corners of mouth, prominent ear crease-left ear | Deep-set eyes, thin upper lip, triangular face | − | Flat nasal bridge, upturned nose |
| Other genetics investigations | | | | | | | |
| Karyotype | normal | normal | normal | NA | 46,XY(t;9;18)(p13;q12.2) | NA | normal |
| Chromosomal microarray | duplication 22q11.2 inherited from the healthy mother | − | − | normal | arr[hg19]4q34.3(178,557,799–179,142,775)x3 (small gain on chromosome 4 in a non-disease associated region) | arr[hg19]2p16.3(51,080,824–51,193,164)x1 (intronic deletion of NRXN1) | arr[hg19]10q11.21(43,555,634–43,626,143)x3 (contains the entire coding region of RET) |
| Gene testing | − | FMR1 negative | FMR1 negative | WGS identified VUS in TUBB2B, c.43G>A; p. (Gln15Lys) | normal Fragile X testing, WES identified the variant c.2380 C>T; p.(His794Tyr) in LZTR1 paternally inherited | NRXN1 sequencing negative | PTPN11, SMN1 deletion, DNA methylation for Prader-Willi/Angelman syndrome, neuromuscular multi-gene panel: negative |

*Nomenclature HGVS V2.0 according to mRNA reference sequence NM_053002.5. Nucleotide numbering uses +1 as the A of the ATG translation initiation codon in the reference sequence, with the initiation codon as codon 1.
**Table 2.**
Comparison of different phenotypes associated with *MED12, MED13, MED13L* and *MED12L* variants.

OSMKB: Ohdo, syndrome, Maat-Kievit-Brunner type. To note that intellectual disability was classified as mainly mild to moderate (+) or mainly moderate to severe (++) . Others signs were considered as very frequent (+), occasional (+/−) or absent/rare (−).

|                  | MED12 | MED13L | MED13 | MED12L |
|------------------|-------|--------|-------|--------|
| **Growth**       |       |        |       |        |
| Tall stature     | +     | −      | −     | −      |
| Macrocephaly     | +     | +      | −     | −      |
| **Facies**       |       |        |       |        |
| Tall prominent forehead | +  | +      | +     | −      |
| Blepharophimosis | −     | −      | +     | −      |
| Downslanting palpebrae | +  | +      | +     | −      |
| High nasal root  | +     | −      | −     | +      |
| High narrow palate| +     | +      | +     | −      |
| Open mouth       | +     | +      | +     | −      |
| Frontal hair upsweep | −  | +      | −     | −      |
| **Hand**         |       |        |       |        |
| Minor hand anomalies | +  | +      | +     | −      |
| **Neurological** |       |        |       |        |
| Congenital hypotonia | +  | +      | +     | +/−    |
| Intellectual disability | + | +      | ++    | ++     |
| Little or no language | −  | −      | +     | +/−    |
| Hypermusal voice | +     | −      | −     | −      |
| Behavior disturbances | +  | +      | +     | +/−    |
| Autism spectrum disorder | +/− | −      | +/−   | +/−    |
| Agenesis/hypoplasia of corpus callosum | +  | +      | +/−   | −      |
| **Gastro-intestinal** |       |        |       |        |
| Anal anomalies   | −     | +      | −     | −      |
| Chronic constipation | −  | +      | +     | +/−    |

*Genet Med.* Author manuscript; available in PMC 2020 May 22.