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

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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 subcomplexes 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.

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INTRODUCTION

Mediator is a key regulator of gene expression involved in cell growth, homeostasis, development, and differentiation.[1] 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.[2,3] 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.[4] 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.[5] 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). Consent for the publication of photographs (Fig. 1) was obtained for individuals 1, 2, and 5.

Molecular analysis
Institutional review board–approved written informed consent was obtained for all subjects. DNA was extracted from leukocytes according to standard procedures.

Copy-number variants (CNVs) 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 60 K or 180 K [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.
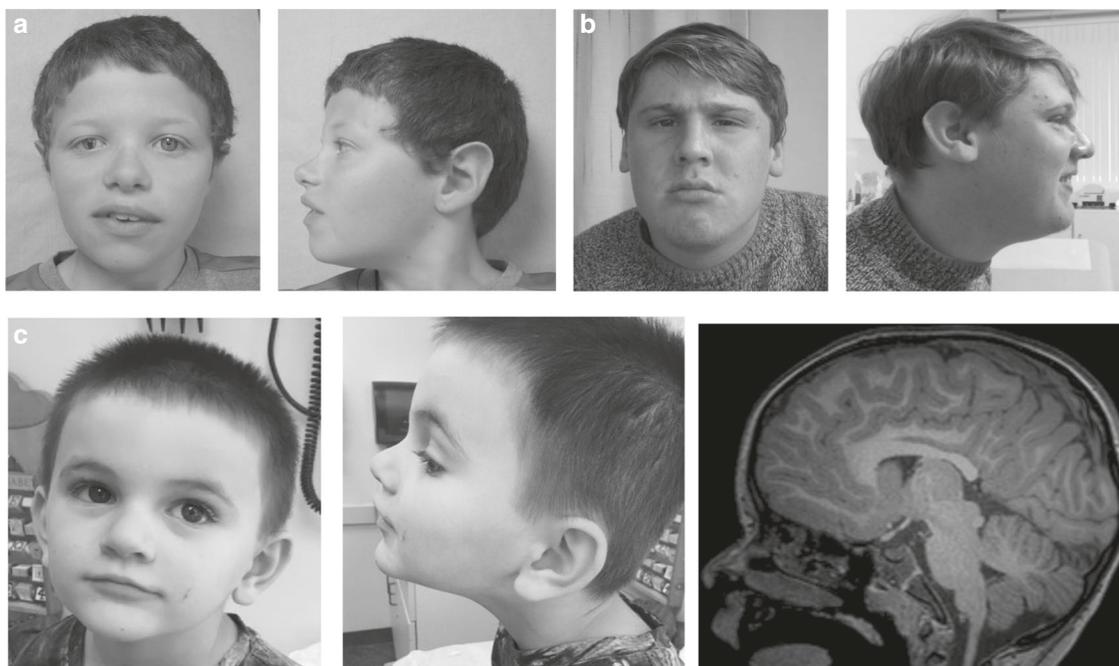
Fig. 1 Photographs and brain magnetic resonance images (MRI) of individuals with variants in *MED12L*. a Individual 1: unilateral ptosis with iris coloboma, hypertelorism, sparse eyebrows, downslanted palpebral fissures, bulbous nasal tip. **b** Individual 2: prominent nasal bridge, short philtrum, everted 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.

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 (National Center for Biotechnology Information [NCBI] build 37.3, hg19). For SNVs, 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 nonradioactive assay as described previously.[20] RRS evaluates the transcription-coupled repair pathway (TC-NER). Cells were plated on coverslips in 6-well plates at a confluence of 7×10^4 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 nonirradiated plates acted as references. After UV irradiation, cells were incubated for 23 hours for RNA Synthesis recovery in Dulbecco's modified Eagle's medium (DMEM) supplemented with fetal bovine serum. Then, after washing with phosphate buffered saline (PBS), cells were labeled with 5-ethynyl-uridine (EU; Invitrogen) for 2 hours. Cells were then washed again with PBS, followed by fixation and permeabilization. The last step involved an azide-coupling reaction and DAPI (6′-diaminido-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 charge-coupled device (CCD) 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 Fig. 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 electroencephalogram (EEG) within normal limits. Brain magnetic resonance imaging

(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 extraneurological 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 Fig. 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, Exome Variant Server (EVS), and 1000 Genome databases. Nonsense variants likely result in the degradation of messenger RNA (mRNA) by nonsense-mediated decay (NMD). 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]

Table 1 Detailed clinical features of the individuals with nucleotide and copy-number variants involving *MED12L*.

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
Variant 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.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	32	39
Pregnancy complications	–	Acute fetal 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 SD)	34 (−1 SD)	21.1 (+0.5 SD)	22.6 (0 SD)	13.3 (−1 SD)
Height (cm/SD)	131.5 (−2.5 SD)	185 (+2 SD)	156 (0 SD)	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 years old)	+ (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	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
Extraneurological abnormalities							
Gastrointestinal anomalies	Chronic constipation, neonatal occlusive	Gastroesophageal reflux	Unilateral inguinoscrotal hernia	–	Feeding difficulties in early infancy, moderate chronic constipation	–	Feeding difficulties (G-tube dependent), gastroesophageal reflux, intermittent constipation

Table 1 continued

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
Congenital malformations	syndrome, encopresia; 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	–	–	–	–	+	–	–
Downslanted palpebral fissures	+	–	–	–	–	–	+
Fullness of the upper eyelids	+	+	–	–	–	–	+
Prominent nasal bridge	–	+	–	+	–	–	–
Bulbous nasal tip	+	–	–	–	+	–	–
Open mouth	–	–	–	–	–	–	+
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 cheekbones, 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)×3 (Small gain on chromosome 4 in a non-disease–associated region)	arr[hg19]2p16.3 (51,080,824–51,193,164)×1 (Intronic deletion of NRXN1)	arr[hg19]10q11.21 (43,555,634–43,626,143)×3 (Contains the entire coding region of RET)
Gene testing	–	FMR1 negative	FMR1 negative	GS identified VUS in TUBB2B: c.43G>A; p.(Gln15Lys)	Normal fragile X testing, ES identified the variant c.2380C>T; p.(His794Tyr) in LZTR1 paternally inherited	NRXN1 sequencing negative	PTPN11, SMN1 deletion, DNA methylation for Prader–Willi/Angelman syndrome, neuromuscular multigene panel: negative

Nomenclature Human Genome Variant Society (HGVS) V2.0 according to messenger RNA (mRNA) reference sequence NM_053002.5. Nucleotide numbering uses +1 as the A of the ATG translationinitiation codon in the reference sequence, with the initiation codon as codon 1.
CNV copy-number variant, EEG electroencephalogram, ES exome sequencing, GS genome sequencing, NA not available, NCBI National Center for Biotechnology Information, OFC occipital frontal circumference, VSD ventricular septal defect, VUS variant of unknown significance, WG weeks gestation.
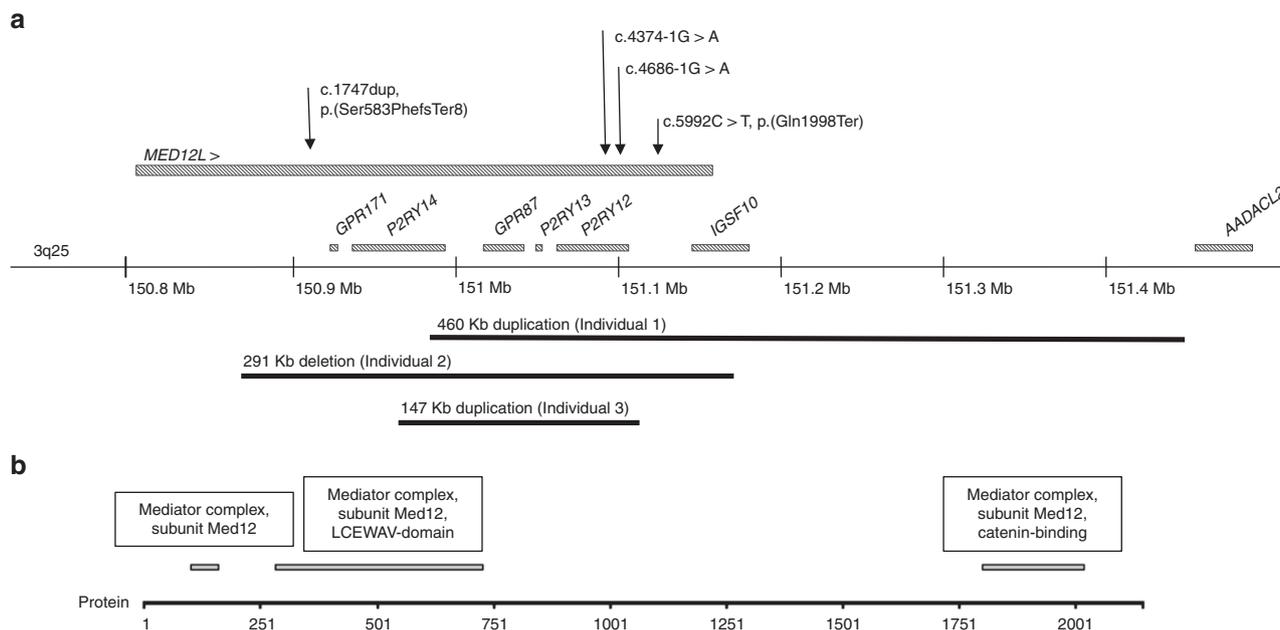
Fig. 2 Mapping of the *MED12L* locus. a Genomic position of *MED12L* variants identified in our series. Upper panel represents single-nucleotide variants. Lower panel represents copy-number variants. **b** Localization of predicted domains of MED12L protein.

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 with 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 with the healthy control, similar to the level observed in a control *MED12* cell line (Fig. 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.[22]

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 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. 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%[23]. The healthy mother of individual 1 carried the same 22q11.2 duplication. Even if we could not exclude a partial role of this duplication, functional data provided and the severity of intellectual disability tended to suggest that *MED12L* CNV had a more important role in the phenotype of individual 1.

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.[22] MED12L is localized to the nucleus and mainly expressed in brain (www.proteinatlas.org). By homology,
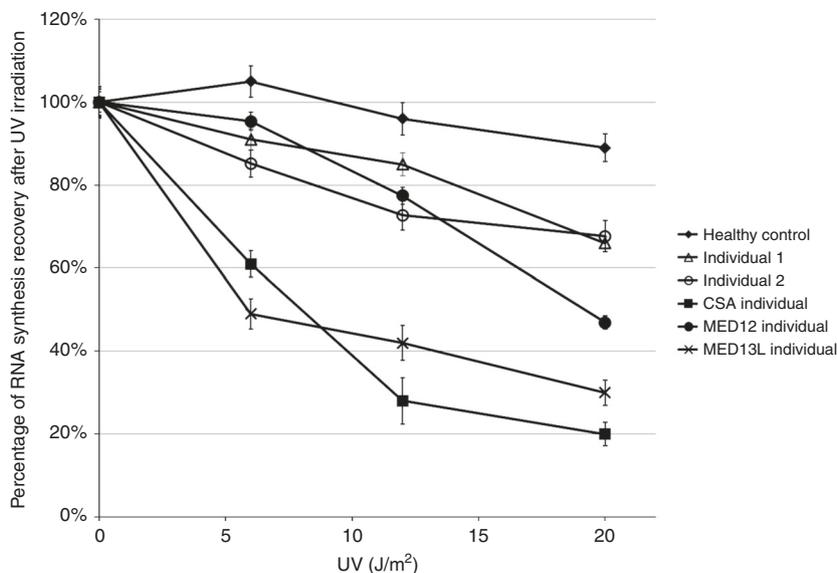
Fig. 3 Recovery of RNA synthesis (RRS) following UV irradiation. Fibroblasts of individuals 1 (△) and 2 (◯) show a moderately decreased level of RRS as compared with the normal control cell line (◆), similar to a *MED12* mutated cell line (●). A *MED13L* mutated cell line (x) shows a more severely decreased 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.

three domains are predicted to be common with MED12, 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/β-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).[24] *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 ($p = 5.25$E-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 preinitiation 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, micro-retrognathia, constipation, and feeding difficulties.[30]

Recently, 13 individuals were described harboring de novo missense and nonsense *MED13* (OMIM 603808) variants. All

Table 2 Comparison of different phenotypes associated with *MED12*, *MED13*, *MED13L*, and *MED12L* variants.

	MED12			*MED13L*	*MED13*	*MED12L*
	Lujan syndrome	FG syndrome	OSMKB			
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	−	−	+	+	+/-	−
Hypernasal voice	+	−	−	−	−	−
Behavior disturbances	+	+	+	+/-	+/-	+
Autism spectrum disorder	+/-	−	+/-	+/-	+/-	+/-
Agenesis/hypoplasia of corpus callosum	+	+	−	+/-	−	+
Gastrointestinal						
Anal anomalies	−	+	−	−	−	−
Chronic constipation	−	+	+	−	+/-	+/-

Intellectual disability was classified as mainly mild to moderate (+) or mainly moderate to severe (++). Other signs were considered as very frequent (+), occasional (+/−), or absent/rare (−).
OSMKB Ohdo syndrome, Maat–Kievit–Brunner type.

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.[13]

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 do not help in pointing to a clinical diagnosis.

Herein, we demonstrate that MED12L fibroblasts for individuals 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 coactivation 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[36,37] and trichothiodystrophy[38] could be due to

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transcriptional defects mediated by variants in the other transcriptional coactivation complexes TFIIH or TFIIE.[39]

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.

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.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-019-0557-3) contains supplementary material, which is available to authorized users.

DISCLOSURE

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