De novo mutations in genes of mediator complex causing syndromic intellectual disability: mediatorpathy or transcriptomopathy?

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BACKGROUND: Mutations in the X-linked gene *MED12* cause at least three different, but closely related, entities of syndromic intellectual disability. Recently, a new syndrome caused by *MED13L* deleterious variants has been described, which shows similar clinical manifestations including intellectual disability, hypotonia, and other congenital anomalies.

METHODS: Genotyping of 1,256 genes related with neurodevelopment was performed by next-generation sequencing in three unrelated patients and their healthy parents. Clinically relevant findings were confirmed by conventional sequencing.

RESULTS: Each patient showed one *de novo* variant not previously reported in the literature or databases. Two different missense variants were found in the *MED12* or *MED13L* genes and one nonsense mutation was found in the *MED13L* gene.

CONCLUSION: The phenotypic consequences of these mutations are closely related and/or have been previously reported in one or other gene. Additionally, *MED12* and *MED13L* code for two closely related partners of the mediator kinase module. Consequently, we propose the concept of a common MED12/MED13L clinical spectrum, encompassing Opitz-Kaveggia syndrome, Lujan-Fryns syndrome, Ohdo syndrome, *MED13L* haploinsufficiency syndrome, and others.

Regulation of gene expression in eukaryotes is an extremely complex process that is controlled by several mechanisms at multiple stages (1). Alterations of transcriptional regulation are involved in the pathogenesis of many human diseases and most of the affected signaling pathways finally target the core transcriptional machinery (2).

MED12 (Mediator Complex Subunit 12) and *MED13L* (Mediator Complex Subunit 13 like) encode two subunits of the macromolecular complex known as "Mediator transcription co-activator complex". The mediator interacts with general transcription factors and RNA polymerase II to regulate transcription. It is the essential coactivator acting as a bridge molecule between transcription factors bound at upstream DNA regulatory elements and the transcriptional machinery. This complex consists of four distinct modules (3,4): the head, middle, and tail modules and all together form the core Mediator Complex that interacts directly with RNA polymerase II and both general and gene-specific transcription factors (5,6), and a fourth "kinase" module that reversibly associates with the core complex which is composed by MED12, MED13, CDK8, and CCNC or their paralogs MED12L, MED13L, and CDK19 (7,8). This module was primarily thought to be involved in transcriptional repression by blocking the binding of the core Mediator Complex to RNA polymerase II because Mediators containing this module are less active than in its absence (4,9). However, there are growing evidences that the kinase module is also involved in transcriptional activation (10).

MED12 gene variants cause at least three different syndromes: Opitz-Kaveggia (FG) (MIM #305450), Lujan-Fryns (MIM #309520), and Ohdo (Maat-Kievit-Brunner type, or OSMKB) (MIM #300895). They all have similar clinical manifestations with multiple congenital anomalies, macrocephaly, hypotonia, and moderate to severe intellectual disability (11). *MED13L* mutations were initially described in patients with congenital heart defect (transposition of the great arteries). However, according to recent studies, the cardiac phenotype is an unclear feature associated with variants of the *MED13L* gene (12–16). Recently, Adegbola and colleagues have described the clinical features caused by *MED13L* deleterious variants. All individuals had intellectual disability and share characteristic facial features, delayed speech and language development, hypotonia, poor coordination, and behavioral problems. None of the patients had a complex congenital heart defect (17).

Here, we report on one patient with a *de novo MED12* mutation and two patients with *de novo MED13L* mutations, not previously described. The clinical features in these patients and in the ones previously reported indicate that mutations in both genes result in similar phenotypes.

RESULTS

All three patients had a normal karyotype and were negative for fragile-X mutation. For the three patients, whole genome dosage analysis was performed by 44K oligonucleotide array

CGH (G4426B Agilent Technologies, Palo Alto, CA). No pathogenic variants were detected.

By applying next-generation sequencing, we identified two novel heterozygous potentially relevant missense variants and one nonsense mutation in the *MED12* or *MED13L* genes (**Figure 1**). Patient 1 showed the *de novo* mutation c.887G>A in the *MED12* gene (NM_005120.2), which causes the aminoacid change arginine to glutamine at position 296 of the protein. Patient 2 carries a *de novo* variant c.5695G>A in the MED13L gene (NM_015335.4), which is predicted to substitute the glycine at position 1899 by arginine. Both substitutions affect highly conserved aminoacids. *In silico* predictions of functional relevance suggested a pathogenic effect for both variants (see **Table 1**) which, together with the fact of occurring *de novo*, are highly suggestive of their pathogenic effect. Patient 3 showed the nonsense mutation c.2524C>T (NM_015335.4), which generates a premature stop codon at exon 14 of the *MED13L* gene. These mutations were not detected in available databases, including the 1000 Genomes database (EMBL-EBI, Hinxton, Cambridge, UK), dbSNP142, the Exome Variant Server (EVS) (Washington, MO), and the Exome Aggregation Consortium (ExAC) (Cambridge, MA). All variants were submitted to the DECIPHER database (Hinxton, Cambridge, UK).

DISCUSSION

In this study, we report one patient with *MED12* and two patients with *MED13L de novo* mutations. All of them display common characteristic facial features, have intellectual disability, speech impairment, motor developmental delay with muscular hypo- or hypertonia and behavioral difficulties. Furthermore, most of the additional clinical features of these patients were previously reported in association to

other mutations in the MEDs genes (see **Table 2**). As it can be observed, there is a wide clinical spectrum among the patients carrying a mutation in *MED12* or among those with mutation in *MED13L*. However, the range of clinical manifestations that can be present when *MED12* or *MED13L* are mutated is strikingly similar. For instance, it is worthy to note that all the features shared by patients 2 and 3, both of them with mutation in the *MED13L* gene (hypotonia, low-set ears, strabismus, hypertelorism, epicantal folds, down-slanting palpebral fissures and depressed nasal bridge, in addition to intellectual disability) are equally reported not only among patients with *MED13L* mutations, but also in the Opitz-Kaveggia or Lujan-Fryns syndromes (see **Table 2**).

Our results support the findings reported by Adegbola *et al.* (17) regarding the absence of congenital heart disease

Table 1. *De novo* mutations detected in *MED12* and *MED13L*

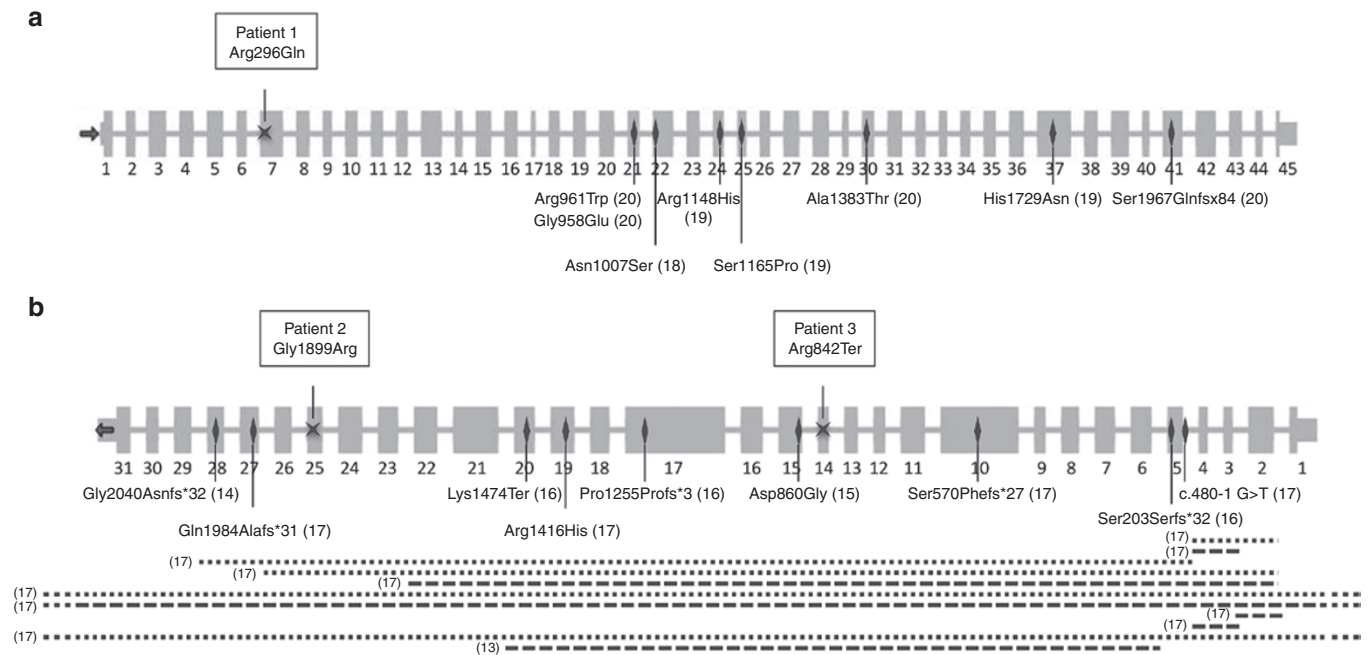|	Patient 1	Patient 2	Patient 3
Position	chrX:70341452G>A	chr12:116413012C>T	chr12:116440853G>A
Gene	*MED12*	*MED13L*	*MED13L*
Coding	c.887G>A	c.5695G>A	c.2524C>T
Transcript	NM_005120.2	NM_015335.4	NM_015335.4
Protein	p.Arg296Gln	p.Gly1899Arg	p.Arg842Ter
Exon	7	25	14
SIFT	0.01	0.0	-
PPH2	1.0	1.0	-
MA	2.74	2.815	-
FATHMM	1.29	-1.87	-
CONDEL	0.527	0.649	-

Figure 1. Distribution of point mutations along *MED12* and *MED13L* genes. Schematic presentation of the *MED12* (**a**) and *MED13L* (**b**) genes (intronic regions are not drawn to scale). The mutations identified in the patients reported here are represented by a cross, and previously published mutations are indicated by a rhomb (if mutations are single nucleotide variant), dotted line (if mutations are duplications) or dashed line (if mutations are deletions).

Table 2. Clinical features of patients with mutations in MED12 and MED13L genes

		MED12 (OHDO syndrome)[a]	MED12 (OPITZ-KAVEGGIA syndrome)[a]	MED12 (LUJAN-FRYNS syndrome)[a]	Patient 1 MED12 Arg296Gln	Patient 2 MED13L Gly1899Arg	Patient 3 MED13L Arg842Ter	MED13L Adegbola et al. (eight patients) (17)	MED13L van Haelst et al. (4 patients) (13)	MED12 Schwartz et al. (seven patients) (18)
Growth paramers	Weight	Low			Low	Low		Short (25%)		Low (28.6%)
	Height		Short	Tall	Short		Short			Tall (57.1%)
Head	Macrocephaly		+	+	Brachycephaly and microcephaly	-	-	Brachycephaly (37.5%)		+ (57.1%) Microcephaly (14.3%)
Face	Prominent forehead		+	+			+	+ (50%)		
	Micrognathia	+	+	+	Microrretrognathia		Retrognathia			+ (71.4%)
	Philtrum	Long and flat	Long	Short and Deep	Long and flat		Short and wide			short (100%)
Ears	Low-set ears		+	+		+	+	+ (62.5%)		
	Hearing loss	+	+			+	-	+ (25%)		
Eyes	Ptosis	+	+			+	-	+ (12.5%)		+ (28.6%)
	Strabismus		+		+	+	+			
	Hypertelorism		+		+	+	+	+ (37.5%)		
	Epicantal folds		+			+	+	+ (25%)		
	Palpebral fissures	BP	Down-slanting		BP	Down-slanting	Down-slanting	Up-slanting (50%)	Up-slanting (75%)	
Nose	Nasal bridge	Wide and depressed		Narrow and High	Split tip and depressed root	Wide and depressed root	Wide and depressed root	Variable		Narrow (57.1%) and High (100%)
	Bulbous nose	+			+		+	+ (62.5%)	+ (75%)	+ (71.4%)
Teeth	Dental crowding		+	+			+ Wide gingival fold	Wide-spaced teeth (25%)		
Genitourinary	Genitourinary anomalies	Cryptorchidism	Cryptorchidism		Cryptorchidism		Unilateral renal agenesis	Cryptorchidism (25%)		
Central Nervous System	Developmental delay	+	+		+	+	+		+ (100%)	
	Intellectual disability	+	+	+	+	+	+	+ (87.5%) Learning disability (12.5%)	Speech delay (100%)	
	Hypotonia		+ (Neonatal)	+	Hypertonia	+	+	+ (50%) Motor delay (100%)	Truncal hypo (50%) Motor delay (100%)	
Behavioral Psychiatric Manifestations	Hyperactivity	+	+	+		+	-	+ (12.5%)		
	Friendly, sociable personality	+	+				+	+ (12.5%)		
	Aggressive behavior			+		+	-	+ (12.5%)		
	Autistic-like behavior			+		+	-	+ (25%)		

BP: blepharophimosis.

[a]Extracted from OMIM clinical synopsis (OMIM #300895; #305450; #309520).

associated with mutations in the *MED13L* gene and, most importantly, emphasizes the clinical similarities between mutations in the *MED12* and *MED13L*.

Based exclusively on the clinical features, patients with mutations in the *MED12* gene were previously classified in three different clinical syndromes, although all of them share in common, to a greater or lesser degree, the same clinical findings: somatic growth disorders (affecting weight and/or height), altered size of the head, micrognathia, philtrum anomalies, some dysmorphic facial features, genitourinary anomalies, abnormal hands and feet, in addition to problems in the central nervous system (agenesis of corpus callosum, seizures, autistic and/or hyperactive behavior, intellectual disability and developmental delay) (reviewed in refs. (11,18,19)). A very similar scenery has been found among the patients with mutations in the *MED13L* gene, who present a wide phenotypic spectrum which basically includes the same clinical features found in association to mutations in the *MED12* (13,17,20,21).

According to Daniels and colleagues (22), the mediator kinase module is formed by MED12, MED13, and CDK8, but also can be formed by their respective paralogs MED12L, MED13L, and CDK19, or by a combination of both. These interactions and the possible formation of different kinase modules can explain the phenotypic spectrum presented by our patients and other patients with *MED12* and *MED13L* mutations. Our hypothesis is that mutations in the different genes of the mediator kinase module may result in similar clinical features, whenever these mutations affect the same protein interactions within the mediator complex, and conversely, mutations in the same gene, affecting other domains implicated in distinct protein interactions, may have different clinical consequences. In other words, mutations affecting MED12 and MED13L are equally responsible for the same phenotypic spectrum, which includes different syndromic entities. All these findings indicate that this transcription mechanism is a complex process involving many protein complexes and that deregulation of one of the components can affect has clinical consequences.

Deleterious variants in other mediator-complex genes have been described: a pericentric inversion that cause haploinsufficiency of *CDK19*, associated with bilateral congenital retinal folds, microcephaly, and mild intellectual disability (23); *MED17* missense variants have been linked to postnatal progressive microcephaly with seizures and brain atrophy (24); *MED23* variants cause autosomal recessive non-syndromic ID (25), and a homozygous *MED25* variant causes autosomal recessive adult onset axonal Charcot-Marie-Tooth neuropathy (CMT2B2, MIM #605589) (26).

In addition to these genes with mutations causing disease, other components of the mediator complex are good candidate genes for pathology. *MED1* gene was disrupted in a mouse model in which null mutants died at an early gestational stage due to heart failure and exhibited impaired neuronal development with extensive brain apoptosis (27). Haploinsufficient mice showed growth retardation, pituitary hypothyroidism and widely impaired transcription in certain organs. On the other hand, a homozygous transversion in the *MED20* gene

was found in two affected sisters from a consanguineous Austrian family, both having infantile-onset basal ganglia degeneration and brain atrophy (28). The patients had a progressive neurodegenerative disorder characterized by severely delayed psychomotor development, spasticity, and dystonia.

Chromatin immunoprecipitation assays showed that depletion of *MED26* led to a reduced phosphorylation of the RNA pol II C-terminal domain and a reduced recruitment of RNA pol II and the SEC components *AFF4* and *CDK9* to both the promoter regions and bodies of the *MYC* and *HSP70* genes (29). The authors hypothesized that *MED26* may function as a molecular switch that in a first instance interacts with the RNA pol II initiation complex through direct interactions with TFIID, and, in a second phase, exchanges TFIID with RNA pol II elongation factors to facilitate productive elongation. TFIID multi-protein complex consists of the TATA binding protein (TBP) and 12 additional TBP-associated factors (TAFs) (30). Variants observed in proteins of this complex (TAF1, TAF2, and TBP) have been implicated in several syndromic forms of intellectual disability and developmental delay (31–34), as well as in X-linked dystonia-parkinsonism (35,36).

Kagey *et al.* (37) disclosed that Mediator and cohesin (SMC1A, MIM#300040) connect, physical and functionally, the enhancers and promoters of active genes in murine embryonic stem cells. Mediator complex interacts with cohesin and form a ring complex that connect two DNA segments because a DNA looping is formed between the enhancers and promoters occupied by Mediator and cohesin. The cohesin-loading factor *NIPBL* (MIM #608667) is associated with mediator-cohesin complexes, providing a mechanism to upload cohesin at promoters. Mutations in the *NIPBL* and *SMC1A* genes cause Cornelia de Lange syndrome, a genetically heterogeneous syndrome characterized by distinct facial features, hirsutism, developmental delay, intellectual disability, and limb abnormalities (38). It is worthy to note that the phenotypic spectrum of Cornelia de Lange syndrome includes clinical features that are widely shared by MEDs syndromes, further suggesting a functionally-related molecular etiology. Some phenotypic features that overlap between MEDs syndromes and Cornelia de Lange syndrome include anteverted nares, long philtrum, high palate, low-set ears, hearing impairment, strabismus, and intellectual disability.

Other protein complex that may interact with mediator complex is the KMT2D complex. This complex has an important role in histone methylation and transcription activation by recruitment of RNA polymerase II complex. Mutations in *KMT2D* cause Kabuki syndrome (39). It is important to note that Patient 1 presented some resemblances with Kabuki syndrome, including microcephaly, absence of palmar digital triradius, highly-arched eyebrows and other facial features (strabismus, long and flat philtrum and cleft palate), genitourinaty anomalies (cryptorchidism), hirsutism, gastrointestinal problems, hypotonia, and intellectual disability. It is thus conceivable that other patients with a phenotype suggestive of Kabuki syndrome could be due to *MED12* or *MED13L* mutations.
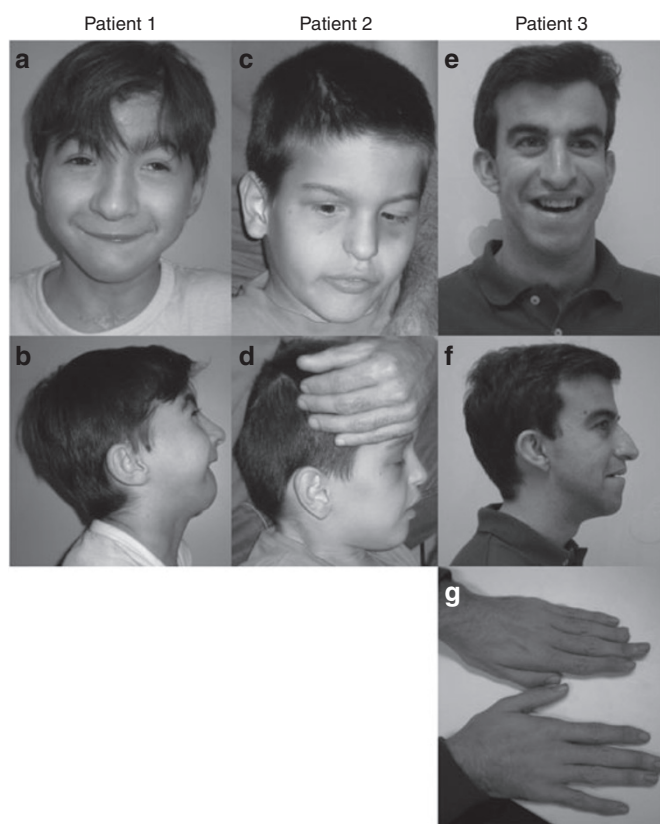
Patient 1   Patient 2   Patient 3

Figure 2. Pictures of the patients illustrating the typical facial features and other relevant clinical features of patients 1 (**a** and **b**) with MED12 mutation, and patient 2 (**c** and **d**) and 3(**e, f** and **g**) with MED13L mutations.

In conclusion, here we present the clinical and laboratory findings of three patients with novel mutations in two distinct genes of the mediator kinase module, *MED12* and *MED13L*. The phenotypic consequences of these mutations are clearly similar and allow us to propose the concept of MED12/MED13L clinical spectrum, encompassing Opitz-Kaveggia syndrome, Lujan-Fryns syndrome, Ohdo syndrome, *MED13L* haploinsufficiency syndrome, and others.

The clinical similarities of MEDs syndromes with other genetic conditions caused by alterations in genes that are components of the initiation machinery of transcription, including the cohesin complex, the TFIID complex or even the KMT2D complex, are also remarkable. Altogether, these clinical similarities caused by different genes, coding for functionally related proteins, fully agree with the recently proposed concept of "transcriptomopathies", a class of disorders that may reflect a global disturbance in transcriptional regulation (40).

METHODS
Subjects
Written informed consent for the study was obtained from all the participants. This study was reviewed and approved by the Institutional Ethics Committee of our hospital and research was carried out in compliance with the Helsinki Declaration.

Genomic DNA from the patients and their parents was isolated from peripheral blood using QIAamp DNA Mini Kit and the QIAcube automated extractor (QIAGEN, Hilden, Germany). DNA quality and concentration were measured using the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Rockland, DE) and was stored at −20 °C.

Patient 1. Patient 1 is a 7-y-old male with multiple congenital anomalies, dysmorphic features and psychomotor delay (DECIPHER no. 323184). He was the second child of non-consanguineous healthy parents. He was born at 37th weeks of gestation by spontaneous vaginal delivery, with a weight of 1,850 g (<10 percentile), length of 45 cm (10–25 percentile) and Occipito-Frontal Circumference of 32.5 cm (25–50 percentile). During pregnancy, oligoamnios and intrauterine growth retardation were observed. At birth, he showed the Pierre-Robin sequence (which required tracheostomy) characteristic facial features, and hypertonia. On clinical examination at 21 mo of age, he had sparse hair, arched eyebrows, ocular hypertelorism, blepharophimosis, bulbous nose with split tip and depressed root, long and flat philtrum, cleft palate, micro-retrognathia, and abnormal ears. He had also brachicephaly and microcephaly, absent digital triradius, bilateral cryptorchidism, overlapping fourth toe, and sandal gap with a subcutaneous fold. At neurological examination he showed psychomotor delay: he was not able to hold his head until 6 mo of age, unable to sit until 14 mo and at 21 mo his speech was absent. magnetic resonance imaging and electroencephalogram were reported as normal. Several dismorphic features were considered reminiscent of Kabuki syndrome. In the last examination at age 7 y, he had a weight of 19 kg (3–10 percentile), height of 114 cm (3–10 percentile), and Occipito-Frontal Circumference of 49 cm (<3 percentile). He had strabismus, astigmatism, high grade of hypermetropia (6 diopters), a small open mouth, chronic constipation, and hirsutism (**Figure 2**). Neurologically, a moderate-severe intellectual disability was noticed, with a very limited use of expressive language and a hyperkinetic behavior.

Patient 2. Patient 2 is an 8-y-old male with multiple congenital anomalies, dysmorphic features and psychomotor delay (DECIPHER no. 323183). He was the second child of non-consanguineous healthy parents. He was born at 35th weeks of gestation, with a weight of 1,880 g (10–25 percentile), length of 43 cm (10–25 percentile), Occipito-Frontal Circumference of 31 cm (25–50 percentile), and hypotonia. During pregnancy, increased nuchal translucency and intrauterine growth retardation were noticed with normal fetal karyotype. He was delivered by cesarean section due to alteration in Doppler-echography. Twenty-four hours after birth, he developed respiratory difficulties that required intubation for 6 d, stayed at intensive care unit for 15 d and hospitalization in the neonatal unit for 1 mo. On physical examination at 17 mo of age, he had asymmetric face, strabismus, left eye ptosis, ocular hypertelorism, downslanting palpebral fissures, bilateral epicanthus, wide and depressed nasal root, tented upper lip with frequent drooling, and low set ears (**Figure 2**). He also had unilateral hearing loss, patent ductus arteriosus, corrected at 12 mo of age, and atopic dermatitis. Neurological examination showed psychomotor delay, as he did not have head control until 7 mo of age, unable to sit until 17 mo, and absence of speech at 17 mo. His behavioral disturbance fitted into the autism spectrum disorder with auto-aggression. Electroencephalogram did not show relevant anomalies. Magnetic resonance imaging showed a prominence of subarachnoid space, predominantly frontal, ventriculomegaly and mega cisterna magna. Signs of periatrial hyperintense areas coexisting with dilated perivascular spaces of Virchow-Robin were considered a normal variant of terminal areas of myelination.

Patient 3. Patient 3 is a 24-y-old male affected with multiple congenital anomalies, dysmorphic features and psychomotor delay (DECIPHER no. 323185). He was the first child of non-consanguineous healthy parents. He was born at 40th weeks of gestation by cesarean section, with a weight of 2,690 g (10–25 percentile), length of 48 cm (25–50 percentile), and Occipito-Frontal Circumference of 35 cm (75–90 percentile). During pregnancy, no problems were detected. Since birth, he had hypotonia with feeding problems. Neurological examination

showed psychomotor delay, he did not had head control until the age of 12 mo old and started to walk at 26 mo. On physical examination at 14 y old, he had hypertrichosis, frontal bossing, low-set hair, synophrys, strabismus, downslanting palpebral fissures, epicanthus, bulbous nose, wide and depressed nasal root, narrow palate, wide gingival fold, abnormal implantation of teeth, low-set ears, and retrognathia (Figure 2). Parents referred that he always had chewing difficulties, but normal swallowing. He also had growth retardation, long and thin fingers, kyphosis, pes cavus deformity, joint hyperlaxity, one café-au-lait spot and unilateral renal agenesis. Mild intellectual disability was reported. In the last physical examination at 24 y of age, he had long face with prominent maxilla and retrognathia, big nose, small ears, middle grade of myopia (5 diopters) and hirsutism. He is able to read and write simple sentences.

Capture Array Design, Next-Generation Sequencing, and Analysis Pipeline

A custom SureSelect oligonucleotide probe library was designed to capture 19,878 coding exons of 614 pathogenic and 642 candidate genes associated with intellectual disability (manuscript in preparation). The design includes all the transcripts reported for each target gene in different databases (RefSeq, Ensembl, CCDS, Gencode, VEGA). The SureSelect DNA Standard Design Wizard (Agilent Technologies) was used for probe design with a 2X tiling density and a moderately stringent masking. A total of 71,994 probes, covering 5.073 Mbp (99.48% coverage of targets), were synthesized by Agilent Technologies (Santa Clara, CA). Sequence capture, enrichment, and elution were performed according to the manufacturer's instructions. The libraries were sequenced on an Illumina HiSeq 2000 platform with a paired-end run of 2×90 bp, following the manufacturer's protocol to generate at least a $100\times$ effective mean depth.

Variation calling was performed with the DNAnexus platform (DNAnexus, Mountain View, CA) through the following pipeline: Fasq paired reads were aligned to the reference human genome UCSC hg19 using the BWA-MEM algorithm from the BWA software package. Mappings were deduplicated using Picard, realigned around sites of known indels, and their quality was recalibrated by looking at covariance in quality metrics with frequently observed variation in the genome. After recalibration, variants were called with the GATK Unified Genotyper module. This pipeline follows the Broad Institute's recommendations for best practices in variant calling. Variants on regions with low mappability or variants in which there was not at least one sample with read depth ≥ 10 were filtered out. Annotation of nucleotide variants was performed by the Ion Reporter Software (Life Technologies, Carlsbad, CA).

Prioritization of Variants

To evaluate the putative clinical impact of the variants, the following criteria were applied: (i) an allele frequency <0.01 in the 1000 genomes or EVS databases; (ii) stop gain, frameshift and splicing variants were *a priori* considered as most likely to be pathogenic; (iii) for missense mutations, amino acid conservation and prediction of pathogenicity (SIFT, Polyphen-2 and Grantham); (iv) a *de novo* occurrence (dominant inheritance), the presence of two mutant alleles in the same gene, each from a different parent (recessive inheritance), or maternal inheritance of X-linked variants; (v) the absence of the variant in other samples (in-house database); (vi) phenotypic consistency with clinical signs associated to mutations in the same gene when available. To evaluate the possible effect of synonymous or intronic variants in gene splicing, we used the Human Splicer Finding web tool.

Validation by Sanger Sequencing

Relevant variants were resequenced by Sanger sequencing. After PCR amplification from DNA of the patient and his/her parents using specific primers, bidirectional sequencing was performed using the BigDye Terminator v1.1 kit following manufacturer's protocol on an ABI PRISM 3500 automated sequencer (Life Technologies). All primers for amplification and sequencing were selected with exon-primer (primers and PCR conditions are available on request).

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