Clinical Case Reports

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CASE REPORT

Nonsyndromic X-linked intellectual deficiency in three brothers with a novel *MED12* missense mutation [c.5922G>T (p.Glu1974His)]

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Key Clinical Message

X-linked intellectual deficiency (XLID) is a large group of genetic disorders. *MED12* gene causes syndromic and nonsyndromic forms of XLID. Only seven pathological mutations have been identified in this gene. Here, we report a novel mutation segregating with XLID phenotype. This mutation could be in favor of genotype–phenotype correlations.

Keywords

Intellectual deficiency, *MED12*, mutation, X-exome sequencing, X-Inactivation, X-linked.

Introduction

X-linked intellectual deficiency (XLID) is a widely heterogeneous group of genetic disorders that involves more than one hundred genes. MED12 (Mediator of RNA polymerase II subunit 12) is a member of the mediator complex which is involved in the regulation of the majority of RNA polymerase II-dependent genes [1, 2]. *MED12* gene has been shown to cause syndromic and nonsyndromic forms of XLID [3]. So far, only seven different germline mutations of this gene have been reported in patients with X-linked intellectual deficiency (XLID), associated with various clinical features.

A common missense mutation – p.Asn1007Ser (c.3020A>G) was found in different families with Lujan syndrome (OMIM 309520), whereas two different missense mutations – p.Arg961Trp(c.2881C>T) and p.Gly958Glu (c.2873G>A) have been found in patients with Opitz-Kaveggia syndrome (OMIM 305450) [4, 5]. Those two syndromes are clinically distinct but they share some clinical aspects, including mild-to-moderate intellectual deficiency (ID), common behavioral patterns, some dysmorphic features, and dysgenesis of the corpus callosum which is frequently associated with these two syndromes [6]. Three different mutations p.Arg1148His(c.3443G>A), p.Ser1165-Pro(c3493T>C), and p.His1729Asn(c.5185C>A) have been subsequently found to cause Ohdo syndrome, Maat-Kievit-Brunner type (OMIM 249620) [7] Which is characterized by ID and dysmorphic features including blepharophimosis, ptosis, small mouth, and a round face with characteris-

tic nose. In addition to these missense mutations, a single frameshift mutation – c.5898dupC – has been reported in the *MED12* gene, in a family including 10 male patients affected with severe to profound nonspecific ID [8].

Here, we report on a novel missense mutation of the *MED12* gene, identified through parallel sequencing of all X-chromosome exons, in three brothers with severe non-syndromic intellectual deficiency and mild dysmorphic features.

Patients and Methods

The family included three affected brothers with severe ID and one unaffected brother. All family members have been clinically evaluated. Cognitive assessment was achieved in patient II-1 and II-3 using the Wechsler Intelligence Scale Child version four (WISC-IV). Skeletal radiography and brain magnetic resonance imaging (MRI) was performed in patient II-3. Informed consent for genetic studies was obtained from parents, according to the French bioethics law.

DNA was extracted from peripheral blood using the standard procedure of phenol chloroform method [9]. Purity and concentration were assessed by NanoDrop ND-1000 Spectrophotometer V3-7 (Thermo Fisher Scientific, Wilmington, DE). DNA from patients II-3 and II-4 was included in a next-generation sequencing project for XLID patients in our institute using SOLiD 5500 sequencer (Life technologies, Grand Island, NY). Five micrograms of DNA were enriched by micro droplet PCR procedure (Raindance technology, Billerica, MA) to target 11,575 exons. Sorting and calling of SNP/InDel were performed using SAMTOOL and GATK softwares. Novelty was assessed by filtering the variants against a set of polymorphisms that are available in public databases such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 genomes (http://browser.1000genomes.org/in-dex.html), Exome Variant Server (http://evs.gs.washington.edu/EVS/), and ExaC (http://exac.broadinstitute.org/). Only nonsynonymous variants or changes affecting splice sites were analyzed. All sequence variants were prioritized by scoring phylogenetic conservation and functional impact (SIFT and Polyphen-2). Candidate variants were selected and confirmed by Sanger sequencing, using the 3500XL Genetic analyzer and the Big Dye cycle sequencing Kit of Applied Biosystem technology. Forward (5′CAGCTCTTCCTACGGTTTGC3′) and reverse (5′ACTTTCAGGCACAGGCTTTCC3′) primers were designed to amplify exon 41 of the *MED12* gene. On the other hand, exon 8 of the *OGT* gene was amplified by forward and reverse primers respectively (5′GCATTAC CAGCCATTAGGC3′/5′CTGCTTTCCCTCTACTATCATG C3′).

X-chromosome inactivation study was carried out in patient I-2 (Mother), according to the method of methylation-sensitive PCR and fragment-length analysis of androgen-receptor CAG repeat polymorphism [10].

Results

Case report

Patient I-1

This 40-year-old female had been adopted during childhood. Family history is unknown. Language was delayed. She had no dysmorphism. She attended school until the age of 16. She has been performing odd jobs since then. She married a nonrelative husband. Three of her four children had developmental delay and ID.

Patient II-1

This male patient was the elder (Fig. 1), he was born at term, after a normal pregnancy. Birth weight was 3.7 kg (+0.7SD), birth height was 50 cm (mean), and birth head circumference was 37 cm (+1.63SD). Apgar score was 10 over 10 minutes. Early psychomotor development was delayed. Walking started after the age of two. At the age of 9 he was still incontinent. He could make a few short sentences. He could eat without help but needed help for the other daily tasks. He had very poor social interactions. He was restless and aggressive. He had severe myopia. At the age of 9, height was 123.5 cm (–1.33 SD), weight was 23 kg (–2 SD), Head circumference was 53 cm (mean). IQ was 40. Facial dysmorphic features included a long narrow face, a high forehead with frontal hair upsweep, a high nasal bridge, and a long philtrum. Fingers and toes were long and thin. The nails of the 5th toes were hypoplasic.

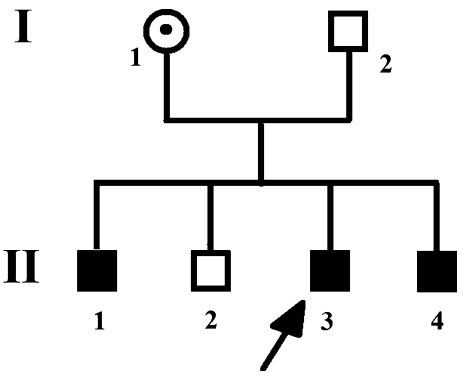

Figure 1. Pedigree of the family. Black symbols indicate intellectual deficiency due to the MED12 mutation. The arrow shows the index case. Circle with a centered black dot represents symptomatic carrier female.

Patient II-2

This 12-year-old male was the second of a sibling of four (Fig. 1), he had normal psychomotor development and no dysmorphic features. He followed his schooling suitably.

Patient II-3

This male patient was the third of a sibling of four (Fig. 1), he was delivered at term, after a normal pregnancy. Birth weight was 3.36 kg mean), birth height was 48 cm (–1 SD), and birth head circumference was 34 (-1 SD). Apgar score was 10 over 10 min. Head holding was acquired after the age of 6 months, sitting at 14 months, and walking at 36 months. At the age of 7 years, weight was 15.7 kg (–2.3 SD), height was 110 cm (–2 SD) and the head circumference was 51 cm (+1 SD). He was severe intellectually impaired with global IQ below 35. Language skills were limited to 10 words; he had problems and difficulties in performing routine daily living tasks. He had stereotypic movements of hands. He had no other abnormal neurological feature. Dysmorphic features included a long narrow face with a high forehead, frontal hair upsweep, mildly down-slanting palpebral fissures, flat malar area, high nasal bridge, a long philtrum, and a small mouth. He was myopic without strabismus (Fig. 2A,B). He had a clinodactyly of the fifth finger and a flat foot (Fig. 2C,D). He did not experience any seizure episode. An EEG recording was performed at 7 years old and was normal. Brain MRI, performed at the age of 10 years, and did not reveal any alteration (Fig. 2E,F). Metabolic and endocrine analyses were normal; karyotype and array-CGH did not show any pathogenic chromosomal imbalance.

Patient II-4

This male patient was born at term of an uneventful pregnancy. Apgar score was 10 over 10 min. Birth weight was 3.5 kg (mean), birth length was 53 cm (+1.5 SD), and birth head circumference was 35 cm (mean). Early psychomotor development was delayed. Walking was achieved at the age of four. He could repeat a few words. At that age 4 years, cognitive evaluation showed severe ID (performance IQ = 30). He was aggressive and restless He had no obvious dysmorphic feature.

Molecular genetics

Sequencing of X-exome from patient II-4 and patient II-2 identified two missense variants that could be considered as potentially pathogenic according to our filters.

The first variant was a c.5922G>T substitution in exon 41 of the *MED12* gene (GenBank accession number: NM_005120) leading to the substitution of a highly conserved Glutamine to a Histidine (p.Gln1974His). This mutation was absent from public databases of control individuals (Exome Variant Server, 1000 genomes, dbSNP135, and ExaC) and in > in-house 200 X-exomes of index patients from other XLID families. This mutation was confirmed by Sanger sequencing in the three affected boys as well as in the mother but was absent in the unaffected brother. This substitution was predicted to be deleterous by SIFT (score: 0) and possibly damaging by Polyphen-2 (score: 0.642) softwares. Predictions with Mutation Taster were in favor of a disease-causing variant (p value: 1). X-chromosome inactivation pattern was not skewed in the mother (60%;40%).

The second variant was the c.955G>A/p.Ala319Thr of the *OGT* gene (GeneBank accession number: NM_181672.2).

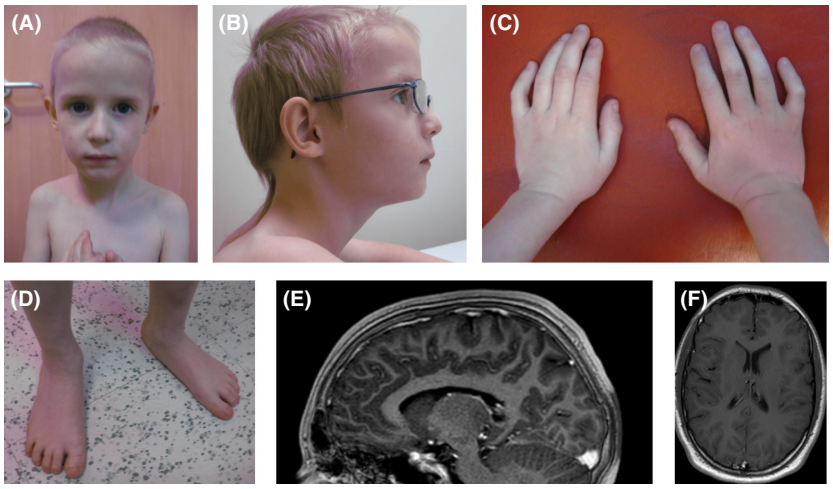

Figure 2. Photographs and brain MRI of patient II-3. (A, B): Facial features include long narrow face with a high forehead, frontal hair upsweep, mildly downslanting palpebral fissures, high nasal bridge, a long philtrum, and a small mouth. (C, D): Clinodactyly of the fifth finger of both hands and flat foot. (E, F): Midsagittal (E) and axial (F) brain MRI sections showing normal corpus callosum.

This variant has not been reported in public databases of control individuals (Exome Variant Server, 1000 genomes, dbSNP135, and ExaC data set) and in >200 X-exomes. In silico predictions are not in favor of a potentially pathogenic role. The *OGT* gene variant co-segregated with *MED12* mutation in the three affected males. It was not found in the nonaffected brother.

Discussion

X-exome sequencing applied to the XLID family reported here brought out two novel missense variants that could be considered as potentially pathogenic. The two variants (c.955G>A-*OGT*) and (c.5922G>T-*MED12*) were confirmed by Sanger sequencing. Both segregated concomitantly with the pathological phenotype of the three affected boys. The two genes are closely located on the X chromosome and are likely to be in Linkage Disequilibrum.

OGT encodes the O-Linked N-acetyl-Glucosamine transferase. It locates on the Xq13.1 band. It has been reported to regulate proteins involved in chromatin remodeling [11] and targets a wide range of intracellular proteins which protect cells from the damaging effects of metabolic stress [12]. Alteration of the *OGT* gene has been shown to play a role in several pathogenic processes which are involved in diabetes, in cancer, in neurodegerative disorders as well as in autism [13]. Many proteins which are involved in neuronal communications, synaptic transmission, and synaptic plasticity are O-GlcNAcylated [14], suggesting an important role for this modification in brain function. Beyond the involvement of *OGT* in chronic human diseases including diabetes, cardiovascular disease, neurodegenerative disorders, and cancer [13, 15, 16]. O-GlcNAcylation was reported to modulate protein phosphorylation and regulates several cellular signaling and functions, mainly in the brain [17]. So far, in terms of brain disorder, the *OGT* gene has been cited only in Alzheimer's disease [18]. However, up to now, no mental disability involvement of this gene has been highlighted and no mutation of *OGT* has been reported, so far, to cause a monogenic disorder. Despite the fact that we could not rule out a role for this mutation in the phenotype of the present family, we gave more consideration to the *MED12* variant.

MED12 is a part of a protein complex involved in the regulation of the majority of RNA polymerase II-dependent genes. It encodes mediator of RNA polymerase II transcription subunit 12 [1, 2]. *MED12* gene mutations alter cell-fate decisions and leads to a variety of pathologic conditions including developmental defects and cancer [19, 20]. Up to now seven different pathological mutations (Fig. 3) have been reported within *MED12* gene and have been associated with ID and a wide clinical variability [6, 8, 21]. Despite clinical variability, patients shared a common core of clinical features, including ID, behavior disorder, and some dysmorphic features. Patients from the family reported here also share these common features (Table 1). However, neither corpus callosum dysgenesis which is a major criterion for LS/FGS nor congenital malformations such as imperforate anus that were commonly delineated in FGS were noticed in the present family. Likewise, blepharophimosis which is constant in X-linked Ohdo syndrome was not detected in this study. Nevertheless, patient (II-3) of this family had a chronic constipation. Severe constipation was reported in FG and OSMBK syndromes (one patient with OSMBK syndrome had Hirshprung disease), and in one patient of the family that was studied by Lesca et al. [8, 21, 22].

On a molecular level, the MED12 C-terminal region harboring all pathological missense mutations found to date interacts with GLI3 protein a Sonic Hedgehog (SHH) signaling effector [23]. It was suggested that mutations disrupt the gene-specific association of MED12 with a second Mediator subunit, CDK8, identified to be a suppressor of GLI3 transactivation activity. Dysregulated GLI3-dependent SHH signaling contributes to phenotypes of individuals with cognitive impairment by modulating the specification of neuronal cell fates and further reveals a basis for the gene-specific manifestation of pathogenic mutations in a global transcriptional coregulator [3].

OGT gene and *MED12* are adjacent, both are muted and they segregate concomitantly in all infected patients, they may have significant effects on neurodevelopment.

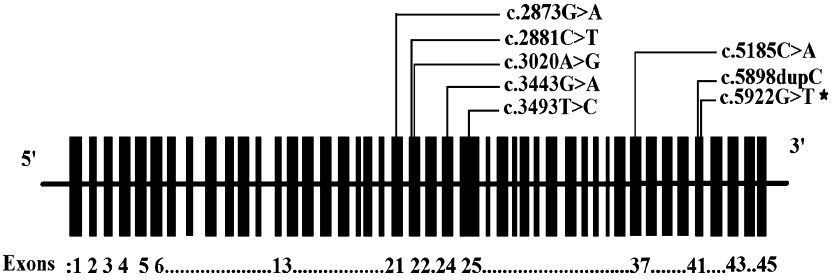

Figure 3. Schematic representation of *MED12* cDNA. The boxes represent the 45 exons. They are numbered from 1 to 45. The position and type of all mutations involved in Intellectual Deficiency which are collected from literature are shown in the upper part. The novel mutation described in this study is shown by an asterisk (*).

Table 1. Comparison of the clinical features of the patients with different mutations of the *MED12* gene.

	FG syndrome Mutations	Lujan syndrome	OSMKB	Lesca et al. [8]	Present study
	p.Gly958Glu p.Arg961Trp Exons	p.Asn1007Ser	p.Arg1148His p.Ser1165Pro p.his1729Asn	Non syndromic[a] p.Ser1967Glnfsx84	p.Gln1974His
Major clinical features	21, 22	22	24, 25, 37	41	41
Intellectual disability	+	+	+	+	+
Behavioral disorders	+	+	+	+	+
No language	–	–	+	+	+
Hypernasal voice hypotonia	–	+	–	–	–
Macrocephaly	+	+	–	–	–
Agenesis of corpus callosum	+	+	–	–	–
Tall stature	–	+	–	–	–
X skewed inactivation	–	–	–	–	–
Affected females	–	–	–	+	+
Anal anomalies	+	–	–	–	–
Constipation	+	–	+	–	–
Hypertelorism/telecanthus	–	–	–	–	–
Strabismus	+	+	+	–	–
Blepharophimosis	–	–	+	–	–
Downslanting palpebrae	+	+	+	–	–
Micrognathia/retrognathia	+	+	+	–	–
Long narrow face	–	+	–	+	+
Triangular face	–	–	+	–	–
Tall prominent forehead	+	+	+	+	+
Facial coarsening	–	–	+	–	–
Frontal hair upsweep	+	–	–	–	–
Thick alae nasi	–	–	+	–	–
High nasal root	–	+	–	+	+
High narrow palate	+	+	+	–	–
Dental crowding	+	+	–	–	–
Maxillary hypoplasia	+	+	–	+	+
Open mouth	+	+	+	+	+
Small ears	+	–	+	–	–
Horizontal palmar creases	+	–	–	–	–
Syndactyly	+	–	–	–	–
Polydactyly	–	–	–	–	–
Thin habitus	–	–	–	+	+

[a]Lesca et al. [8].

The c.5898dupC, reported by Lesca et al. was located in the same exon as the mutations found in the present study [8]. It had been found in a family including 10 males affected with severe to profound XLID and with cognitive impairment in a high proportion of heterozygous females. The phenotype of those patients was very similar to that of the present family and patients were considered as having nonspecific XLID. Another feature shared by these two families is the presence of intellectually impaired heterozygous females. This was not reported in families with previously reported missense *MED12* mutations, in which males usually had milder intellectual impairment.

Conclusion

The involvement of the *MED12* gene in intellectual disability has been confirmed. There are few pathological mutations known in this gene. So far only seven mutations have been identified. Our novel missense variant (c5922G>T) was the eighth. It segregates with the pathological phenotype in all affected patients**.** Although the number of *MED12* mutations reported to date is too limited to draw any definite genotype–phenotype correlations, mutations in different regions might cause more severe cognitive impairment or more pronounced dysmorphic features.

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Conflict of Interest

None declared.

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