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MED12 missense mutation in a three-generation family. Clinical characterization of *MED12*-related disorders and literature review

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ABSTRACT

Mutations in *MED12* gene have been described in association with syndromic and non-syndromic X-linked intellectual disability (XLID). Up to date at least three distinct XLID syndromes have been described: FG syndrome, Lujan-Fryns syndrome (LS) and Ohdo syndrome (OSMKB). In the last years, thanks to the massive use of next generation sequencing techniques (NGS) it has been possible to discover at least 16 others *MED12* mutations and to expand the phenotype of *MED12*-related disorders. Here we report three subjects from a large non-consanguineous family presenting with a mild to severe ID, important speech delay, behavior problems, dysmorphic facial features and hearing loss. NGS allows us to detect the *MED12* missense variant c.3883C > T (p.(Arg1295Cys)) carried by the three patients. This variant has been reported in 2016 by Hu et al. in one family from a big cohort of XLID families. This clinical report contributes to expanding the phenotype associated with *MED12*-mutations.

1. Introduction

MED12 gene is located at Xq13.1 and encodes one of the subunits of the large Mediator complex. This multi-protein complex has more than 30 subunits arranged in four different modules and his role is critical in RNA polymerase II transcription (Donnio et al., 2017). In particular, MED12 protein is involved in the regulation of the majority of RNA polymerase II-dependent genes (Shin et al., 2008) (Wang et al., 2013). Mutations in this gene have been identified in patients affected by syndromic and non-syndromic X-linked intellectual disability (XLID) (Zhou et al., 2012).

Up to date at least three distinct XLID syndromes caused by germline mutations in *MED12* have been described. FG syndrome [OMIM 305450] has been the first syndromic intellectual disability to be linked to *MED12* gene. It is caused by two recurrent mutations, c.2881C > T (p.(Arg961Trp)) and c.2873G > A (p.(Gly958Glu)) and is characterized by moderate to severe intellectual disability (ID), behavioral abnormalities, hyperactivity, relative macrocephaly, hypotonia, broad thumbs, constipation or imperforate anus and corpus callosum hypoplasia or agenesis (CCA) (Opitz and Kaveggia, 1974) (Risheg et al., 2007) (Graham et al., 2008) (Graham and Schwartz, 2013). Lujan-Fryns syndrome [OMIM 309520] is caused by a *MED12* recurrent missense mutation c.3020A > G (p.(Asn1007Ser)) and it is characterized by mild to moderate ID, psychosis, tall stature with a marfanoid habitus, long hands with hyper extensible digits, macrocephaly, high narrow palate, hyper nasal voice and CCA. (Lujan et al., 1984) (Schwartz et al., 2007). Three different *MED12* missense mutations have been found to cause the third syndrome, Ohdo syndrome (OSMKB) [OMIM 300895] c.3443G > A (p.(Arg1148His)), c.3493T > C (p.(Ser1165Pro)) and c.5185C > A (p.(His1729Asn)). This syndrome is characterized by an ID associated to distinctive facial features, such as blepharophimosis, ptosis, coarse face with a characteristic nose (thick alae nasi) and a small mouth (Maat-Kievit et al., 1993; Verloes et al., 2006; Vulto-van Silfhout et al., 2013).

In the last years, thanks to next generation sequencing techniques (NGS) it has been possible to discover new *MED12* mutations and to expand the phenotype of *MED12*-related disorders.

In 2013, Lesca et al. identified a novel *MED12* frameshift mutation, c.5898dupC (p.(Ser1967Glnfs*84)) in a five generations family presenting with non-syndromic profound ID. Cognitive impairment was also observed in seven heterozygous females (Lesca et al., 2013). Another novel *MED12* missense mutation, c.5922G > T (p.(Glu1974His)), has been reported in 2015 by Bouazzi et al. in three brothers showing severe non-syndromic ID and mild dysmorphic features. In this family, as already described by Lesca et al., a mild phenotype characterized by borderline ID and language delay was found in their heterozygous mother (Bouazzi et al., 2015). In 2016 Prontera et al., reported on a novel c.2312T > C (p.(Ile771Thr)) *MED12* missense mutation, detected by Whole Exome Sequencing (WES) in a family with two males and a female affected by ID and presenting a phenotype that overlap in part with the ones described by Lesca et al. and Bouazzi et al. (Prontera et al., 2016). Other variants have been reported through the last years; Callier et al. found a possibly pathogenetic *MED12* missense mutation, the c.3884G > A (p.(Arg1295Cys)) in a 18-years old male with mild ID and marfanoid habitus. The mother was heterozygous for the mutation and showed a milder phenotype (Callier et al., 2013). Yamamoto and Shimojima described a novel *MED12* mutation, c.3067A > G (p.(Ile1023Val)), in a patient affected by non-syndromic ID (Yamamoto and Shimojima, 2015). Tzschach et al., identified a *MED12* variant, c.2444G > A (p.(Arg815Gln)) in a family showing moderate ID, short stature and microcephaly (Tzschach et al., 2015). Langley et al. reported a new maternally inherited missense mutation in *MED12*, c.4147G > A (p.(Als1383Thr)) in two male siblings with global developmental delay, failure to thrive, penile chordee, microcephaly, blepharophimosis and facial dysmorphism (Langley et al., 2015). Prescott et al. found the c.1862G > A (p.

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(Arg621Gln)) in a 10-year old boy presenting with mild to moderate ID associated to a severe Pierre Robin sequence and a fetus showing a severe micrognathia (Prescott et al., 2016). Caro-Llopis et al. described a male patient with ID, Pierre-Robin sequence, blepharophimosis, microcephaly, chronic constipation and cryptorchidism, carrying the *de novo* mutation c.887G > A (p.(Arg296Gln)) in the *MED12* gene (Caro-Llopis et al., 2016). Narayanan and Phadke reported the c.4832G > A (p.(Arg1811His) mutation in a 5-year old male with ID, speech delay, ptosis, facial dysmorphism and short stature. (Narayanan and Phadke, 2017). Finally, three new *MED12* gene mutations has been reported by Charzewska et al. in three families. The c.3271G > A (p.(Glu1091Lys)) mutation was found in a family with three affected male cousins showing ID, developmental delay and facial dysmorphism; all carrier females had learning difficulties. The c.2861T > G (p.(Val954Gly)) mutation was detected in four affected brothers with ID and developmental delay, relative macrocephaly, facial dysmorphism, strabismus and sociable personality. The c.4111C > T p.(Pro1371Ser)) mutation was identified in a male patient with a severe ID, severe hypotonia, dysmorphic features, macrocephaly, ptosis, Hirschsprung disease, congenital heart and brain defects, seizures, (Charzewska et al., 2018).

Here we describe a three generations family where two cousins and the maternal uncle show a mild to severe ID and dysmorphic facial features. The three subjects carry the *MED12* missense variant c.3883C > T (p.(Arg1295Cys)) which has been detected by sequencing a NGS panel. This variant has been reported in 2016 by Hu et al. in one family from a big cohort of 405 XLID unresolved families (Hu et al., 2016). Only recently Charzewska et al. have provided clinical details of this specific family (Charzewska et al., 2018).

2. Patients

This three generations family consists of 14 subjects (Fig. 1). The proband (III-6), his cousin (III-4) and his uncle (II-3) present a mild to severe ID. The three patients have healthy, non-consanguineous parents. Both the subjects III-6 and III-4 had a sister who died in infancy and the mother of subject III-6 had three miscarriages in the first trimester of pregnancy. In particular, subject III-7 died at 20 months of age for a pneumococcal sepsis. She was reported to present sensorineural hearing loss (SNHL) and a facial phenotype similar to her brother. Subject III-2 died at 4.5 months of age due to a severe respiratory distress after a bronchiolitis. Unfortunately, we did not have more clinical information regarding the two girls and DNA samples were not available.

Subject III-6 has a 12-year old living sister, III-5, who presented a double renal reflux in infancy, with good results after corrective surgery. She is reported to be a high potential child and to have dyspraxia and dysgraphia. She has attended a specialized school for high potential children and she did not complain with any medical problems.

Subject III-4 has two healthy older brothers, III-1 and III-3. The second one presented only with a double renal reflux in infancy corrected by surgery at the age of 3 years old.

The three heterozygous females did not undergo cognitive evaluation but the clinical evaluation did not detect any problem and they presented with a normal global development. They show a mild dysmorphism: prominent forehead, hypertelorism and down-slanting palpebral fissures (Fig. 2). Subject I-1 has a severe asymmetric hearing loss, sensorineural in the right hear and mixed in the left one. She has been using hearing aids since the age of 46 years.

2.1. Patient 1

The proband (III-6) is a 10-year-old boy who presents an important speech delay, dyspraxia and dysgraphia. Walking was acquired at 17 months. The IQ is borderline; the WPPSI-III done in 2013, when he was 6 years old, showed a verbal IQ of 98, performance IQ of 87 and a processing speed IQ of 69. He has a moderate sensorineural hearing loss in the left ear and mixed in the right side discovered when he was 3 years old. He was delivered at term after an uneventful pregnancy. Birth length was 52 cm (+1 SD), weight was 3.970 kg (+1 SD) and head circumference was 36,5 cm (M). At birth he presented with hypotonia and laryngomalacia with stridor. He had a left cryptorchidism and a partial cleft palate with velopharyngeal insufficiency. Clinical evaluation at the age of 10, shows tall stature, height is 153 cm (+3 SD), weight at 41 kg (+3 SD), normocephaly, head circumference is 55,5 cm (+2 SD), long narrow face, prominent forehead, sparse eyebrows, hypertelorism, long and down-slanting palpebral fissures, high nasal root, malar hypoplasia, bifid uvula, velopharyngeal insufficiency with hypernasal speech, high narrow palate, micrognathia, shorts hands and feet and flatfeet (Fig. 3). He has three "café au lait" spots on the neck, abdomen and thorax and one depigmented spot on the left side of the thorax. The proband presents also strabismus and hypodontia due to dental agenesis of 6–8 teeth. He has a characteristic behavior and a friendly and cooperative personality. Brain MRI and CT scan of the temporal bone were normal. Vestibular testing showed left hyporeflexia. The skeletal X-ray examination did not detect bone malformation and the bone age was normal. Echocardiography and EKG were normal. The proband underwent various genetic tests, all resulted negatives: FISH 22q11, Fragile-X analysis, standard karyotype, Array-CGH, *MID1* gene sequencing and NGS panel for Marfan and Marfan-like syndromes. A *GJB2/GJB6* and *STRC* genes analysis was also done trying to understand if hearing loss was part of the syndrome or if it would be due to a separate genetic cause, but we did not find any mutation.

2.2. Patient 2

Subject III-4 is the maternal first cousin of the proband. He is an 18-year-old boy who presents with a mild ID. He was born at term after a normal pregnancy; birth weight was 2910 kg (−1 DS) and birth length

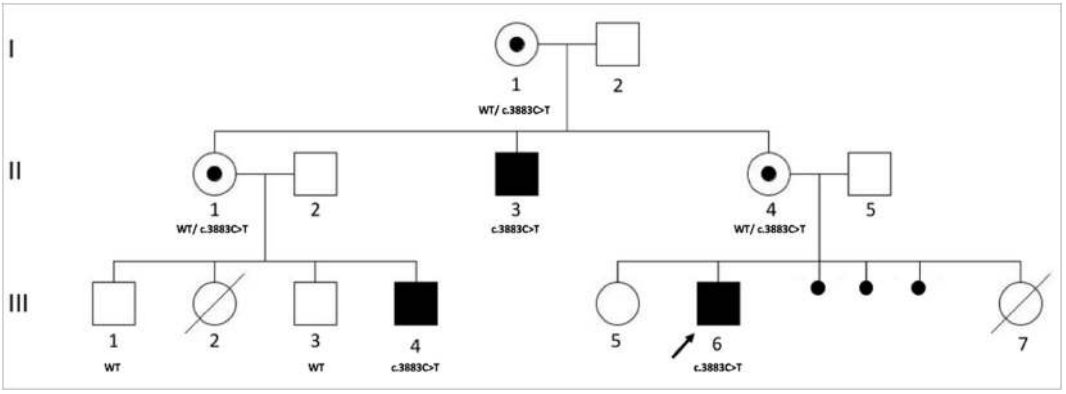

Fig. 1. Pedigree of the three generations family. Arrow indicates the proband. WT = *MED12* wild type.

Fig. 2. Facial dysmorphism of the three female carriers (the 70-years-old proband's grand-mother, I-1, his 44-years-old mother, II-4 and his 49-years-old maternal aunt, II-1). Note the high forehead, hypertelorism and down-slanting palpebral fissures.

was 47 cm (−2 SD). Apgar score was 5/8/9. He presented with a respiratory distress due to a laryngo-tracheo-broncho-malacia and neonatal hypotonia. He had a unilateral left cryptorchidism and a velopharyngeal insufficiency. He presented also a moderate mixed hearing loss in the left ear, discovered when he was 6 years old probably due to recurrent ear, nose and throat infections during his childhood. He did not get a hearing test recently.

Subject III-4 had delayed developmental milestones; sitting was acquired at 10 months, walking at 17 months and the speech was severely delayed. He has a severe dyspraxia. The WPPSI-III done in 2007 at the age of 8 years, showed a verbal IQ of 87, performance IQ of 69 and a full-scale IQ of 76.

We firstly met him at the age of 18. Clinical evaluation shows a relative macrocephaly, height is 172 cm (M) and head circumference is 58 cm (+2 SD), long narrow face, prominent forehead, sparse eyebrows, hypertelorism, long and down-slanting palpebral fissures, high nasal root, malar hypoplasia, high narrow palate, micrognathia (Fig. 3). He presented a hypernasal speech due to his velopharyngeal insufficiency. He has one “café au lait” spot on the left lumbar region and one depigmented spot on the right hypochondriac region. He presents also with an alternant convergent strabismus and astigmatisms and hypodontia due to dental agenesis of 8 teeth. He has a friendly and cooperative personality but he is reported to have severe behavior problems characterized by anxiety, recurrent bursts of aggressiveness and psychosis. He is treated with methylphenidate since he was 7 years old. Brain MRI showed small areas of leukomalacia on the periventricular white matter. The metabolic tests, Fragile-X analysis and standard karyotype were negatives.

2.3. Patient 3

Subject II-3 is the maternal uncle of the proband and subject III-4. He is a 46-year-old man born at term to unrelated parents who presents with a moderate ID. Birth weight was 3.980 Kg (+1 SD) and length was 51 (+1 SD). After birth he was reported to have respiratory problems, stridor and hypotonia. He presented with craniosynostosis corrected by surgery at the age of 6 months, bilateral cryptorchidism and cleft palate. He showed a developmental delay with poor verbal skills. Cognitive evaluation has not been done for this subject. Clinical evaluation, done at the age of 46, shows a relative macrocephaly, height is 183 cm (+1 SD) and head circumference is 59 cm (+2 SD), long narrow face, prominent forehead, sparse eyebrows, hypertelorism, long and down-slanting palpebral fissures, high nasal root, malar hypoplasia, high narrow palate, micrognathia (Fig. 3). He presented a hypernasal speech due to his velopharyngeal insufficiency. He has a moderate to profound, bilateral sensorineural hearing loss (SNHL) discovered in the adulthood but he did not perform any audiometry before. He has a friendly and cooperative personality but he is reported to have behavior problems characterized by anxiety and psychosis. No genetic test has been done in the past for this patient.

3. Molecular genetics

Written informed consent including consent for the genetic analysis and the publication of images was obtained for all the members of the family, according to the French bioethics law.

Array-CGH analysis has been performed in the DNA of both cousins and a Fragile-X syndrome has been excluded by *FMR1* expansion analysis.

Next Generation Sequencing panel targeting 35 genes involved in overgrowth associated with ID was performed in the patient III-6. Genomic DNA was extracted from 5 ml of peripheral blood leukocytes with standard methods. Agilent SureSelect libraries were prepared from 2 μg of genomic DNA sheared with a Covaris S2 Ultrasonicator. NGS sequencing was done in our institute using a NextSeq (Illumina Inc., San Diego, CA). Paired-end sequence datasets were aligned against human genome (hg19, UCSC Genome Browser). Variant calling of SNV and small indels was realized by samtools, GATK and Varscan. Variant annotation was based on Ensembl human database (GRCh37 release). In order to identify potential causative variations, a consecutive filtering approach has been applied. Variants present in dbSNP135 and Exome Sequencing Project Databases (ESP), Exome Variant Server (EVS, ESP6500SI-V2), 1000 Genomes (May 21, 2011) and the GnomAD database (http://gnomad.broadinstitute.org, October 2017) with a frequency higher than 1% has been excluded. Of the remaining variants, the ones affecting splicing sites or coding regions (nonsense, missense, insertion or deletion) has been selected. In order to be classified as probably pathogenic, a novel variant needed to present the characteristics determined by the international guidelines of the American College of Medical Genetics (ACMG) Laboratory Practice Committee Working Group.

Familial segregation on the DNA samples of 7 family members, has been done by PCR and Sanger sequencing (Fig. 4).

X-chromosome inactivation (XCI) profile analysis on the blood cells of the three female carriers of our family was performed using methylation-sensitive PCR and fragment-length analysis of the androgen-receptor CAG repeat polymorphism.

Fig. 3. Facial dysmorphisms of the three affected subjects of the family (the 10-years-old proband, III-6, his 18-years-old maternal cousin, III-4 and their 46-years-old maternal uncle, II-3). Note the long face, high forehead, hypertelorism, long and down-slanting palpebral fissures short philtrum, bulbous nose and long rotated external ears.

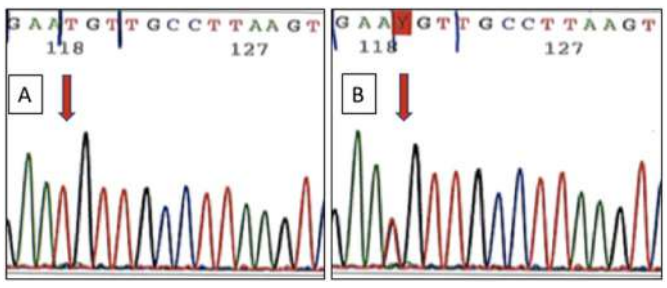

Fig. 4. Chromatogram showing (A) hemizygous c.3883C > T, p.(R1295C) in the proband (III-6) and (B) heterozygous c.3883C > T, p.(R1295C) in his mother (II-4).

Table 1
Comparison of clinical findings between the three well-known *MED12*-related syndromes and the three families showing a variation in position 1295 of MED12 protein. Clinical features common to the three families are marked in bold.

Feature category	Clinical features MED12 mutations	FG syndrome	LS	OSMKB syndrome	Callier et al.		Charzewska et al.	Present case
		Arg961Trp, Gly958Glu	Asn1007Ser	Arg1148His, Ser1165Pro, His1729Asn	Arg1295His		Arg1295Cys	Arg1295Cys
General	Sex affected	M	M	M	M	F	M	M
	ID	+	+	+	+	+	+	+ (3/3)
	Developmental delay	+	+	+	+	-	+	+ (3/3)
	Speech delay	-	-	-	-	-	-	+ (3/3)
	Hypernasal speech	-	+	-	+	-	-	+ (3/3)
	Tall stature	-	+	-	+	+	+	+ (1/3)
	Thin habitus	-	+	+	+	+	+	NA
Central nervous system	**Neonatal hypotonia**	+	+	+	-	-	+	+ (3/3)
	Corpus callosum agenesis (CCA)	+	+	-	-	-	+	-
	Spasticity with joint contracture	+	-	-	-	-	-	-
	Seizures and EEG abnormalities	+	+	+	-	-	+	-
Craniofacial	Macrocephaly	+	+	-	-	-	-	+ (2/3)
	Microcephaly	-	-	+	-	-	-	-
	Long narrow face	+	+	-	-	-	+	+ (3/3)
	Triangular face	-	-	+	-	-	-	-
	Coarse facies	-	-	+	-	-	-	-
	High prominent forehead	+	+	+	-	-	+	+ (3/3)
	Sparse eyebrows	-	+	-	-	-	+	+ (3/3)
	Hypertelorism	+	-	+	-	-	+	+ (3/3)
	Downslanting palpebral fissures	+	+	+	+	-	-	+ (3/3)
	Upslanting palpebral fissures	-	-	-	-	-	+	-
	Ptosis	-	+	+	+	-	-	-
	Blepharophimosis	-	-	+	-	-	-	-
	Maxillary hypoplasia	+	-	+	-	-	-	-
	Malar hypoplasia	-	-	-	+	-	-	+ (3/3)
	Prominent nasal bridge	-	+	-	-	-	+	-
	Anteverted nares	-	-	-	-	-	+	-
	Thick alae nasi	-	-	+	-	-	-	-
	High nasal root	+	+	-	-	-	-	+ (3/3)
	Short philtrum	-	+	-	-	-	-	-
	High narrow palate	+	+	+	-	-	+	+ (3/3)
	Cleft lip/palate	-	-	+	-	-	-	+ (2/3)
	Micrognathia	+	+	+	-	-	-	+ (3/3)
	Dental anomalies	-	+	+	+	-	+	+ (2/3)
	Small ears	+	-	+	+	-	+	-
	Large ears	-	-	-	-	-	-	-
Ophthalmologic	Nystagmus	+	-	-	-	-	-	-
	Strabismus	+	+	+	-	-	+	+ (2/3)
Auditory	Hearing loss	-	-	+	-	-	-	+ (2/3)
Musculoskeletal	Skeletal anomalies	+	-	+	-	-	+	-
	Long superextensible digits	-	+	-	-	-	-	-
	Broad thumbs and halluces	+	+	-	-	-	-	-
	Singe palmar crease	-	-	-	-	-	+	-
Cardiopulmonary	Cardiac abnormalities	+	+	+	-	-	+	-
Gastrointestinal	Gastrointestinal anomalies	+	-	+	-	-	-	-
	Chronic constipation	+	-	+	-	-	-	-
Genitourinary	**Cryptorchidism**	+	-	+	-	-	+	+ (3/3)
	Inguinal hernia	+	-	-	-	-	+	-
Behavior	Characteristic behavior	+	+	+	-	-	-	+ (3/3)
	Psychosis	-	+	-	-	-	-	+ (2/3)

4. Results

Data analysis from the ID with tall stature panel performed on the proband, showed the presence of the rare variation NM_005120.2: c.3883C > T; p.(Arg1295Cys) in the exon 28 of *MED12* gene. Segregation analysis showed that the two other affected males (subject III:4 et II:3) were hemizygous and that their healthy mothers were heterozygous (Fig. 4).

The c.3883C > T; p.(Arg1295Cys) *MED12* variation, leads to the substitution of an arginine to a cysteine. Arginine in position 1295 is highly conserved across species from humans to drosophila. This missense variant was predicted to be deleterious by Polyphen2, SIFT and CADD. It has not been reported in the public available databases such as 1000 genomes and gnomAD. This mutation has been submitted to the public reference database LOVD (Individual #00230642).

The three female carriers presented a skewed XCI profile in their blood cells. In particular it was 20:80 in the subjects I:1, 10:90 in II:1 and 13:87 in II:4.

5. Discussion

Up to date three well defined phenotypes with a strong genotype-phenotype correlation, have been described in association with *MED12* mutations. FG syndrome is characterized by ID, macrocephaly, broad thumbs, imperforated anus, CCA and hypotonia. LS is an X-linked intellectual disability syndrome associated with marfanoid habitus, macrocephaly, congenital hypotonia and hypernasal voice. OSMKB syndrome is characterized by ID, blepharophimosis, ptosis, long philtrum, micrognathia and hearing loss.

In recent years several associations of clinical features related to new *MED12* mutations have been described but, up to date the limited number of patients does not permit to define new distinct phenotypes.

Lesca et al., Bouazzi et al. and Prontera et al., reported three families where both hemizygous males and heterozygous females were affected (Bouazzi et al., 2015; Lesca et al., 2013; Prontera et al., 2016). The males presented with a more severe phenotype characterized by profound ID, poor verbal skills and behavioral problems. In 2013 Callier et al. detected a new *MED12* variant in a male with mild ID, developmental delay, tall stature, long and thin habitus, malar hypoplasia, down slanting palpebral fissures, ptosis, dental abnormalities, small ears and nasal speech (Callier et al., 2013). In this family the mother presented with a milder phenotype. Yamamoto and Shimojima described a male patient affected by non-syndromic ID and a long narrow face (Yamamoto and Shimojima, 2015). Patients with a new mutation from the cohort reported by Tzschach et al. presented with moderate ID, short stature and microcephaly (Tzschach et al., 2015). Langley et al. reported a new missense mutation in *MED12* in two male siblings with mild to severe ID, developmental delay, failure to thrive, hypertonicity, penile chordee, microcephaly, blepharophimosis and dysmorphic features (short palpebral fissures, triangular face and hypothelorism) (Langley et al., 2015). Prescott et al. found a new *MED12* mutation in two siblings, a boy showing mild to moderate ID associated to hypotonia, chronic constipation, abnormal eye motility, hearing loss, dysmorphic features (Pierre Robin sequence, triangular face, narrow palate) and a fetus showing a severe micrognathia (Prescott et al., 2016). Caro-Llopis et al. described a *de novo* mutation in the *MED12* gene in a male patient with moderate to severe ID, Pierre-Robin sequence, hypertonia, chronic constipation, blepharophimosis, cleft palate, micro-retrognathia, microcephaly and cryptorchidism (Caro-Llopis et al., 2016). Narayanan and Phadke reported a male patient with mild ID, speech delay, ptosis, hypertelorism, prominent nose and short stature (Narayanan and Phadke, 2017). Recently Charzewska et al. described three new mutations. One was found in three affected male cousins showing moderate ID, developmental delay, hypotelorism, prominent nose, narrow lips, prognathism, narrow and high palate and long hands; in this family all carrier females had learning difficulties. The second mutation was detected in four affected brothers with moderate to severe ID and a severe developmental delay, relative macrocephaly, long face, prominent forehead, bulbous nose, long philtrum, sparse hair, strabismus and sociable personality. The third mutation was identified in a male patient with a complex phenotype characterized by severe ID, severe hypotonia, short stature, cerebral atrophy, corpus callosum hypoplasia, seizures, congenital heart, renal and skeletal defects. In this patient a WES analysis revealed also a *de novo* pathogenic mutation in *PUF60* gene (p.(Arg123Trp)). The authors believe that some of the clinical findings, never reported in probands with *PUF60* mutations, such as macrocephaly, ptosis, Hirschsprung disease, genital hypoplasia and anal anomaly, could fit in the spectrum of *MED12*-related disorders. This patient has also a conductive hearing loss (Charzewska et al., 2018).

We report three affected males from the same family who share some common features with the well described *MED12*-related syndromes, such as tall stature (1 out of 3 patients), long narrow face, prominent forehead, micrognathia in association with neonatal hypotonia, cryptorchidism and ID with poor language skills and behavioral characteristics (Table .1). However, we did not detect corpus callosum dysgenesis or agenesis, which are major criterion for LS/FG syndromes, nor congenital gastrointestinal malformations, such as imperforate anus, or broad thumbs that are frequently seen in FG syndrome. Furthermore, none of the three patients showed blepharophimosis, the typical features of OSMKB. Our patients presented also various degree of hearing loss, subject III-6 and II-3 showed a bilateral SNHL whether subject III-4 had unilateral mixed HL. SNHL has been reported in OSMKB patients and in one of the two patient described by Prescott et al. (2016).

Even if our patients do not match the criteria for the recognized *MED12*-related syndromes we think that their phenotypic features show some similarity to the ones described by other authors in the last years (Table .1). On the contrary the ID in our patients was variable and less severe than the ones described by the others authors.

Moreover, in our family the three female carriers are healthy and they do not present any degree of ID. We only note that they shared some mild facial dysmorphism (high forehead, hypertelorism and down-slanting palpebral fissures) and that subject I-1 presents a severe bilateral asymmetric mixed HL discovered in the adulthood. XCI pattern analysis in their blood cells was skewed and even if we could not determine which allele (mutated or wild-type) was preferentially inactivated, we assume that would probably be the mutated one. In this family we described two girls deceased at very young age; the first one, subject III-2 due to a respiratory distress without further information and the second one, subject III-7 following a pneumococcal infection. DNA for these two subjects was not available so we cannot asses if they were heterozygous for the *MED12* mutation or not. Besides, even if they were carriers, we would not be able to predict if they presented a higher susceptibility to infection due to their carrier status. Subject II-4 presented three early miscarriages but we cannot determine whether they are related or not to *MED12* mutation. However, we note that in the family previously described by Prescott et al. the mother also presented multiple pregnancy losses (Prescott et al., 2016).

In FG, LS and OSMKB syndromes, ID is not a common features in females (Clark et al., 2009; Schwartz et al., 2007; Vulto-van Silfhout et al., 2013), but in some of the families more recently reported, the authors described also a milder phenotype with variable degree of ID in heterozygous subjects. More recently Charzewska et al. described two affected sisters showing developmental delay with ID and speech delay, dysmorphic facial features (high frontal hairline, hypertelorism, epicanthic folds, deep set eyes, high nasal root, very small nares, bulbous nasal tip, short philtrum, small mouth, strabismus and deep set, posteriorly rotated, simple ears) who carried one of the known OSMKB mutation c.3443G > A (p.(Arg1148His)). The variant was inherited from their healthy mother (Charzewska et al., 2018). In order to explain the difference in the female carrier phenotype, XCI in the blood cells of

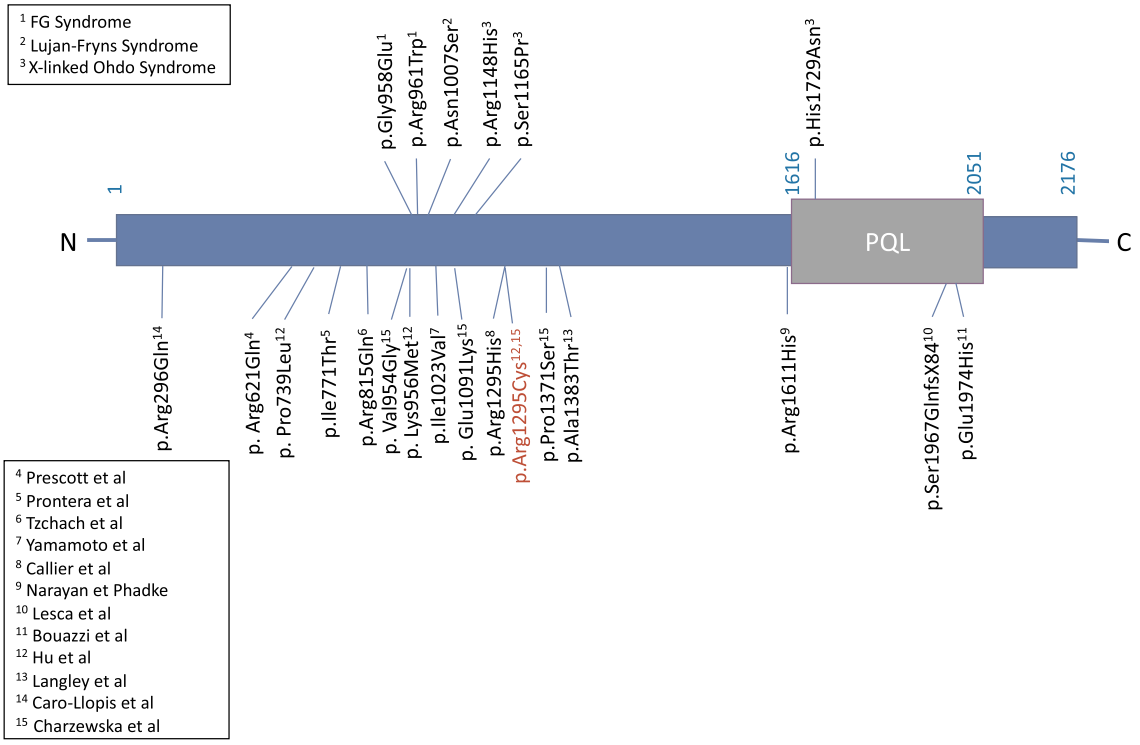

Fig. 5. Schematic representation of the *MED12* primary structure and sites of knowing mutations in the MED12-related disorders (upper side) and in the XLID (bottom side). PQL indicates the domain with a high content of Pro, Gln, and Leu residues, as reported by Kim et al. (Kim et al., 2006). In red is the mutation presented by the family in this study (Figure adapted from Yamamoto and Shimojima, 2015). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

these subjects was carried out. Some of the authors did not find a correlation between their lyonization pattern and the cognitive function (Bouazzi et al., 2015; Charzewska et al., 2018; Lesca et al., 2013; Risheg et al., 2007). Some others described a skewed XCI pattern in heterozygous affected female (Prontera et al., 2016; Vulto-van Silfhout et al., 2013). However, so far it is not clear whether or not skewed XCI is a consistent finding and if it could be used in the future to predict the phenotype in female carriers.

Up-to-date twenty-two pathological *MED12* mutations have been described in literature (Fig. 5). In particular, two recurrent mutations, c.2881C > T (p.(Arg961Trp)) and c.2873G > A (p.(Gly958Glu)) have been reported in 24 patients with FG syndrome (Risheg et al., 2007); the c.3020A > G (p.(Asn1007Ser)) is the recurrent missense mutation shared from all Lujan-Fryns syndrome patients described (Schwartz et al., 2007) and three missense mutations, c.3443G > A (p.(Arg1148His)), c.3493T > C (p.(Ser1165Pro)) and c.5185C > A (p.(His1729Asn)), have been identified in patients presenting the OSMKB phenotype (Vulto-van Silfhout et al., 2013). The remaining sixteen reported variations have been found in patients affected by a variable degree of cognitive impairment, behavior disorders and some dysmorphic features. Among these, there are three *MED12* mutations, c.5898dupC (p.(Ser1967Glnfs*84)), c.5922G > T (p. (Glu1974His)) and c.2312T > C (p.(Ile771Thr)) which have been associated with a more severe phenotype characterized by profound ID and absent or very limited language also in heterozygous females. The authors hypothesized that the presence of mutations with a more severe biological effect (e.g. truncating mutations) could lead to this phenotype. The severity of these mutations can also explain ID in females, even in presence of normal or minor skewed XCI pattern (Bouazzi et al., 2015; Lesca et al., 2013; Prontera et al., 2016).

The c.3883C > T, (p.(Arg1295Cys)) *MED12* missense variant carried by our patients has already been described in 2016 by Hu et al. (2016). The authors performed X-exome sequencing in a big cohort of 405 unresolved families (Hu et al., 2016) with suggestive X-chromosome involvement and at least two affected males for each family. They found three *MED12* segregating likely pathogenic missense variants, p.(Arg1295Cys), p.(Pro739Leu), p.(Lys956Met) and the previously reported c.5898dupC (p.(Ser1967Glnfs*84)) mutation, in three of these families. In this paper the authors did not specify the phenotype for each mutation. We only know that their patients did not match the three well described *MED12*-related syndromes. In particular: 11/11 affected males presented with severe to profound ID and flat malar area; prominent forehead, long and narrow face was found in 10 out of 11 subjects, a high nasal bridge in 9 of them, a short philtrum in 8 of them and a friendly personality in 7 of them. Among the 10 female carriers, cognitive impairment was described in 8 subjects. More recently Charzewska et al. described the clinical details regarding the c.3883C > T, (p.(Arg1295Cys)) variation. The two affected males (the proband and his maternal uncle) presented with ID, developmental delay, tall stature with thin habitus, hypotonia, seizures, corpus callosum agenesis, long narrow face with a prominent forehead, broad nasal bridge, anteverted nares, hypertelorism, upslanted palpebral fissures, sparse eyebrows, strabismus, small ears, high arched palate, dental anomalies, small thorax, cryptorchidism, inguinal hernia and hoarse voice (Charzewska et al., 2018) (Table .1).

In 2013 Callier et al. described another possibly pathogenetic variant in position 1295, the c.3884G > A (p.(Arg1295His)) (Callier et al., 2013). This variant leads to the substitution of an arginine, which is highly conserved between species, with a histidine. The authors described a male patient with mild ID, developmental delay, tall stature, long and thin habitus, malar hypoplasia, downslanting palpebral fissures, ptosis, dental abnormalities, small ears and nasal speech and a carrier mother presenting with a milder phenotype (Table .1). The presence of at least three family showing a similar phenotype, reinforces the idea that the substitution of the arginine in that position of *MED12* gene has to be consider pathogenic.

The clinical and phenotypic variability between the patients described by the different authors trough the last years is very wide, and it

could probably be explained by the multiple functions of the MED12 protein in complex pathways. *MED12* gene which encodes a subunit of the transcription regulating multiprotein Mediator complex, is involved in fact in growth, development, differentiation, extraneuronal gene silencing and signaling suppression (Wang et al., 2013) (Conaway et al., 2005).

Future determination of other detailed genotype-phenotype correlation of *MED12* variants, both in male patients and female carriers are required to clarify the wide spectrum of these disorders. In particular the frequency of hearing loss in the affected males, the presence of miscarriages and predisposition to severe infections in heterozygous females need to be carefully evaluated in order to improve follow up and genetic counseling of these patients.

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Appendix A. Supplementary data

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