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ATRX mutation in two adult brothers with non-specific moderate intellectual disability identified by exome sequencing ☆

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ABSTRACT

In this report, we describe two adult brothers affected by moderate non-specific intellectual disability (ID). They showed minor facial anomalies, not clearly ascribable to any specific syndromic patterns, microcephaly, brachydactyly and broad toes. Both brothers presented seizures. Karyotype, subtelomeric and FMR1 analysis were normal in both cases.

We performed array-CGH analysis that revealed no copy-number variations potentially associated with ID. Subsequent exome sequence analysis allowed the identification of the *ATRX* c.109C>T (p.R37X) mutation in both the affected brothers. Sanger sequencing confirmed the presence of the mutation in the brothers and showed that the mother is a healthy carrier.

Mutations in the *ATRX* gene cause the X-linked alpha thalassemia/mental retardation (ATR-X) syndrome (MIM #301040), a severe clinical condition usually associated with profound ID, facial dysmorphism and alpha thalassemia. However, the syndrome is clinically heterogeneous and some mutations, including the c.109C>T, are associated with a broad phenotypic spectrum, with patients displaying a less severe phenotype with only mild-moderate ID. In the case presented here,

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exome sequencing provided an effective strategy to achieve the molecular diagnosis of ATR-X syndrome, which otherwise would have been difficult to consider due to the mild non-specific phenotype and the absence of a family history with typical severe cases.

1. Introduction

Intellectual disability (ID) affects approximately 1–3% of the general population and is diagnosed upon the concomitant occurrence of three fundamental criteria: an intelligence quotient (IQ) below 70, significant limitations in two or more adaptive skill areas, and the presence of the condition from childhood (before the age of 18 years).

Causes of ID can be either genetic, including chromosomal and single gene disorders, or non-genetic, such as problems during pregnancy, at birth and after birth, as well as poverty and cultural deprivation (Ropers, 2010). Although such environmental influences can cause ID, gene mutation and structural rearrangements of the genome are now considered to be the most important factors in this disorder (Firth and Carter, 2010). Genetic ID can be subdivided into syndromic forms, which are characterized by ID accompanied by either malformations, or dysmorphic features, or neurological abnormalities, and non-syndromic forms, which are characterized by ID without any additional features. A great effort has been made over recent years to improve knowledge on the genetic basis of ID, which has lead to the identification of several genes implicated in both syndromic and non-syndromic forms, the majority of them mapped to the X chromosome (Ropers, 2010). Despite these recent advances, the etiology of ~60% of cases of ID is still unknown. The application of exome sequencing promises to rapidly reduce the number of such unexplained cases, allowing the identification of disease causing variants in both known and yet unknown ID genes (Topper et al., 2011).

We report here on two adult brothers affected by moderate ID and dysmorphic peculiar minor facial anomalies which did not fit a specific syndromic pattern. We exploited the whole-exome sequencing strategy in order to identify the causative genetic defect and found the c.109C>T (p.R37X) mutation in the X-linked alpha thalassemia/mental retardation (ATR-X) syndrome (MIM #301040) causing gene, *ATRX*. This *ATRX* mutation is often linked to a mild and non-specific phenotype. Our study provides evidence of the usefulness of the exome sequencing technique for the achievement of a rapid and certain molecular diagnosis of idiopathic ID cases.

2. Clinical report

2.1. Patient 1 (III-6, Fig. 1b)

Patient III-6 was evaluated at the age of 38 years. He is a male, second born of healthy non-consanguineous Caucasian parents, aged 23 and 25 years at time of conception. First born (III-5) is a healthy male. Third born will be described later as Patient III-7. The family history was otherwise unremarkable and negative for ID or birth defects. A first grade cousin (from the father's side) died at eight months of life, as reported, for meningitis secondary to otitis (Fig. 1a).

The child was spontaneously delivered at term after an uneventful pregnancy. His birth weight was 3200 g (10th centile). Other neonatal auxological parameters are unknown. Growth parameters were reported to be at the lower limits of the normal range until the eighth month of life and then they were regular, while gross motor, cognitive and social milestones were retarded: he walked without support and took his first steps at two years of age and he spoke his first words at the age of three years. At eight months he developed a tonic–clonic seizure in hyperpyrexia. At 2 years, he developed myoclonic absences that were controlled by anticonvulsants. At that time, EEG detected bilaterally widespread cortico-subcortical abnormalities. Cranial Magnetic Resonance Imaging (MRI), performed at 38 years, showed microcrania with a more evident reduction of frontal lobes and moderate cerebellar atrophy, more marked against the worm with extension of sub-arachnoid space in the posterior fossa.

On our evaluation, at the age of 38 years, his auxological parameters were: weight 79 kg (90–97th centile); height 174 cm (25–50th centile); and OFC 56 cm (50–75th centile). He had a long face with a small forehead, bushy eyebrows, prominent nasal root and tip, thickened helix, arched palate and broad

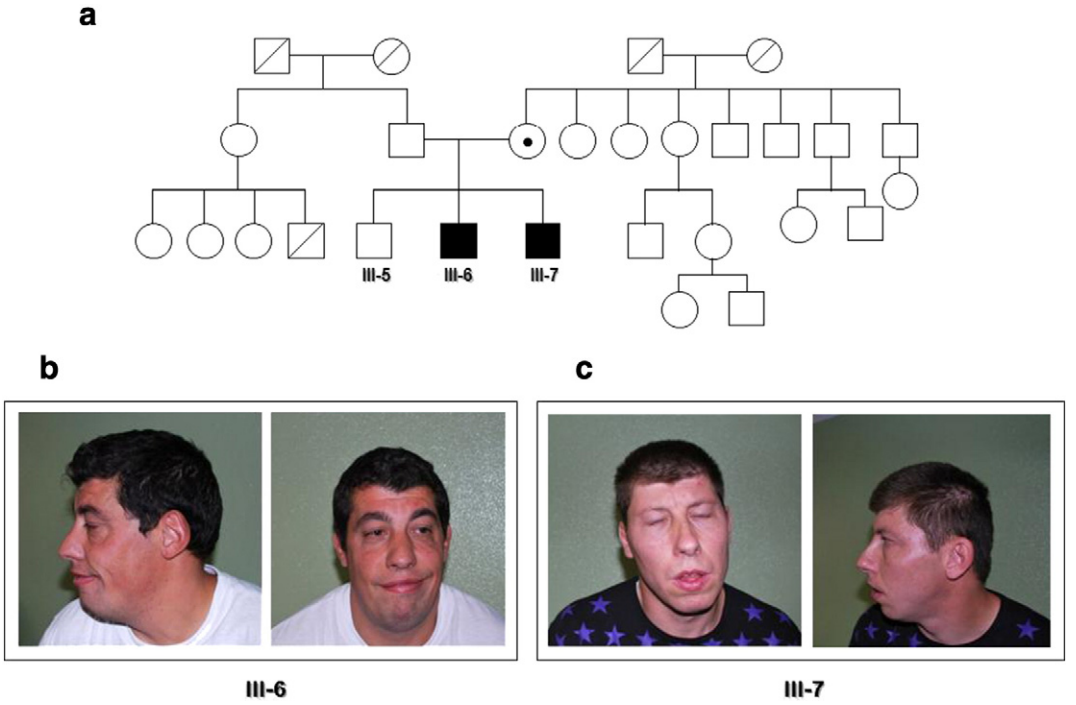

Fig. 1. Family tree (a) and facial features of the two affected brothers (b, c). (a) A first grade cousin (from the father's side) died at eight months of life, as reported, for meningitis secondary to otitis; (b) patient III-6, aged 38 years, exhibiting minor dysmorphic features, including long face with small forehead, bushy eyebrows, prominent nasal root and tip, thickened helix, arched palate, and broad lips; (c) patient III-7, aged 36 years, shows mild microcephaly, a long face with a small forehead, prominent nasal bridge and tip, thickened helix, arched palate, and short neck.

lips (Fig. 1b). In addition, he had brachydactyly of fourth and fifth fingers, stubby first toes, proximal attachment of fourth and fifth toes, and valgus knee. Ophthalmologic examination, as well as cardiac and renal ultrasounds was normal. Aminoacidemia/uria was normal. Neuropsychological evaluation by WAIS-R showed moderate ID with VIQ 53, PIQ 57 and FSIQ 50.

2.2. Patient 2 (III-7, Fig. 1c)

Patient III-7 is case III-6's brother. The child was spontaneously delivered at term after an uneventful pregnancy. His birth weight was 3000 g (10th centile). Other neonatal auxological parameters are unknown. Growth parameters were reported to be at the lower limits of the normal range until the eighth month of life and then they were regular, while gross motor, cognitive and social milestones were retarded: he took his first steps at the age of two years, he walked without support and spoke his first words at three years of age. At eight months he developed a tonic–clonic seizure in hyperpyrexia. At 2 years, he developed myoclonic absences that were controlled by anticonvulsants. At the age of ten years, a neuropsychological evaluation showed a moderate intellectual disability. At 26 years he developed behavioral problems controlled by antipsychotic drugs. Cranial Magnetic Resonance Imaging (MRI), performed at 36 years, showed a microcrania and microcephaly with more evident reduction of frontal lobes.

At time of our evaluation his auxological parameters were: weight 79 kg (90–97th centile); height 175 cm (50th centile); and CC: 53 cm (3rd centile). He had mild microcephaly, a long face with a small forehead, bushy eyebrows, prominent nasal bridge and tip, thickened helix, arched palate, short neck (Fig. 1c), brachydactyly, broad and stubby first toes with proximal attachment, and mild valgus knee. Neuropsychological evaluation by WAIS-R showed moderate ID with VIQ 55, PIQ 54 and FSIQ 49.

3. Materials and methods

Informed consent was obtained from the parents for the molecular analysis and for the reproduction of photographs.

Genomic DNA was isolated from peripheral blood samples using the QIAamp DNA blood mini kit (Qiagen). Array-based Comparative Genome Hybridization (a-CGH) was performed on the Agilent oligo-a-CGH 244K, which includes 236,000 60 nmer long oligonucleotide probes (both coding and non-coding sequences), with an average spatial resolution of ~30 kb.

Exome sequence analysis was carried out on an Illumina HiSeq platform after exome capture using the Agilent SureSelect Human All Exon 50 M kit (BGI Hong Kong Co.). For Sanger sequencing detection of the c.109C>T *ATRX* mutation, blood derived DNA was PCR amplified using *ATRX*-Fw: 5′-GCCTCCTGAGTAGCTGAGAC-3′ and *ATRX*-Rev: 5′-GCTTGCTAATCTGTCATTTTCCA-3′ primers, sequenced using the BigDye Terminator Cycle Sequencing Ready Kit (Life Technologies Corporation), and run on an automated ABI-3130xl DNA genetic analyzer (Applied Biosystems).

4. Molecular results

Routine chromosome analysis and FISH (fluorescent in situ hybridization) analysis with subtelomeric specific clones as well as molecular testing of FMR1 were normal in both patients. In order to rule out subtle genomic rearrangements as the possible cause of ID, an array-based Comparative Genomic Hybridization analysis was carried out, which however revealed no copy-number variations (CNVs) potentially associated with ID.

The subsequent exome sequence analysis showed the presence of the c.109C>T (p.R37X) nonsense mutation in exon 2 of the *ATRX* gene (chr. Xq13) in both patients but not in their healthy brother. Traditional Sanger sequencing confirmed the presence of the mutation in the affected brothers and showed that the mother is a healthy carrier (Fig. 2).

5. Discussion

Mutations in the *ATRX* gene, mapped to Xq13, cause the X-linked alpha thalassemia mental retardation (ATR-X) syndrome (MIM #301040), a rare disease with an estimated prevalence of <1–9/100,000 live

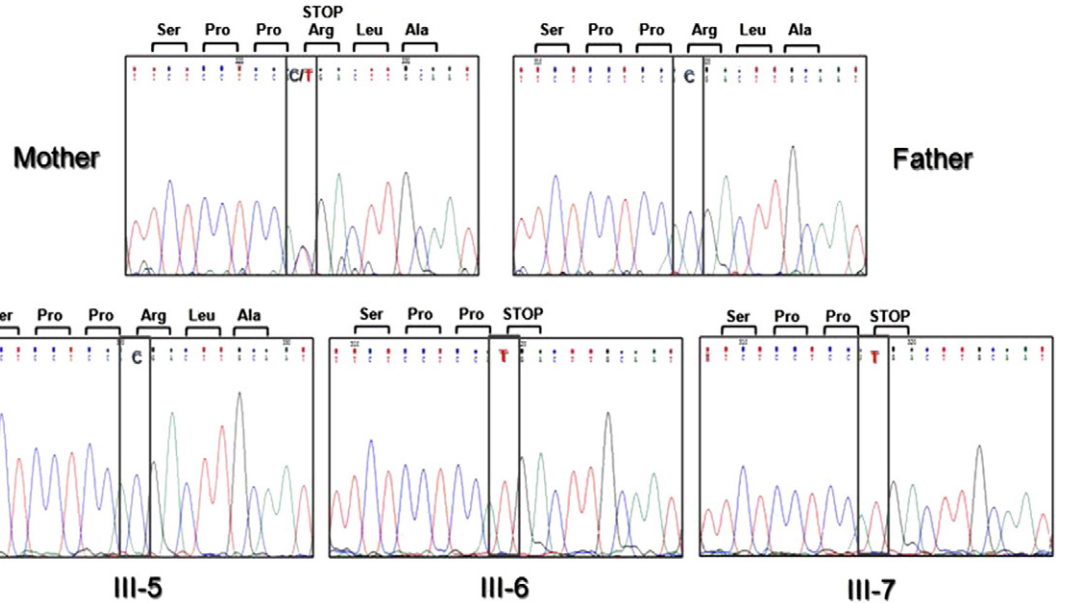

Fig. 2. Detection of the c.109C>T (p.R37X) mutation in the two affected brothers (III-6 and III-7) by sequence analysis of ATRX exon 2. The mutation was also detected in the heterozygous state in their mother.

born males (Gibbons, 2006) and only about 170 cases reported worldwide (Jezela-Stanek et al., 2009). In addition, other phenotypically overlapping conditions, such as Carpenter–Waziri syndrome or Juberg–Marsidi syndrome (Villard et al., 1996; Abidi et al., 1999), are caused by mutations in the *ATRX* gene. *ATRX* spans about 300 kb of genomic DNA, contains 36 exons and encodes a member of the SNF2 family of adenosine triphosphatase/helicases, with a role in chromatin remodeling and gene expression (Tang et al., 2004; Gibbons et al., 2008). The individuals affected by ATR-X syndrome are almost exclusively male and the usual findings are profound developmental delay and ID (present in >95% of cases), genital abnormalities (85%), alpha thalassemia (80%) and a typical facial appearance (Gibbons, 2006). However, the syndrome is clinically heterogeneous and a few mutations, including the c.109C>T variant identified in our cases, are associated with a broad phenotypic spectrum, with patients displaying a less severe phenotype with the absence of typical features of the syndrome and only mild-moderate ID (Gibbons et al., 2008; Yntema et al., 2002). The c.109C>T mutation was first discovered by Guerrini et al. (2000) in a family with two individuals with mild ID and absence of typical features, in whom the ATR-X syndrome was only suspected after it had been clinically diagnosed in two other family members who presented severe ID and the characteristic features of the syndrome. The phenotypic variability linked to this nonsense mutation in *ATRX* exon 2, as well as to other nonsense mutations identified in the *ATRX* gene, can be ascribed to the presence of a residual amount of wild-type protein, maybe by initiation of translation at a downstream methionine or alternative splicing (Howard et al., 2004; Abidi et al., 2005).

In our cases, first evaluated in adulthood, the clinical features overlapped with the phenotypic spectrum of the syndrome solely in the presence of skeletal abnormalities, seizures and, only as far as patient III-7 is concerned, microcephaly. Instead, they lacked the other specific signs of the syndrome, such as specific minor facial anomalies, genital abnormalities and, most importantly, profound ID (Table 1). Moreover, the characteristic facial features of ATR-X syndrome, consisting of upswept frontal hair line, hypertelorism, epicanthic folds, flat nasal bridge, small triangular upturned nose, retracted columella, tented upper lip, everted lower lip and hypotonic facies were mainly absent. In addition to this, since neither patient had symptoms of anemia, the presence of HbH inclusions was not evaluated.

The exome sequencing technique represented here an effective tool for arriving at the molecular diagnosis of ATR-X syndrome, which otherwise would have been difficult to consider due to the relatively mild and non-specific phenotype of the two affected brothers and the lack of a family history with typical severe cases (Guerrini et al., 2000), also avoiding time-consuming screening of candidate genes. Several

Table 1
Comparison of clinical features between previous ATR-X cases and the patients reported here.
Modified from Guerrini et al., Ann Neurol 2000; 47:117–121.

Clinical features	ATR-X syndrome (%)	Patients	
		III-6	III-7
Age		38 years	36 years
Severe/profound ID	95	−	−
Weschler IQ scores			
FSIQ		50	49
Verbal score		53	55
Performance score		57	54
Skeletal abnormalities	90	+	+
Characteristic face	>90	−	−
Hemoglobin H inclusions	85	ne	ne
Neonatal hypotonia	93	−	−
Genital abnormalities	80	−	−
Gut dysmotility	95	ne	ne
Microcephaly	75	−	+
Short stature	65	−	−
Seizures	30	+	+
Cardiac defects	20	−	−
Renal/urinary abnormalities	15	−	−

Legend:
FSIQ: full scale intelligence quotient.
ne: not evaluated.

genes have been identified so far, and many others are likely to be discovered in the near future, which can give rise to both syndromic and non-syndromic forms of ID (Ropers, 2010). The application of exome sequencing to the diagnosis of ID, besides increasing the speed at which unknown ID genes are revealed, will also help to uncover further sporadic or familial cases with mutations in already known causative genes but with non-specific phenotype, thereby improving genetic counseling to the affected families.

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