



Short report

Deletion of the *AP1S2* gene in a child with psychomotor delay and hypotonia

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ABSTRACT

We identified a 495 Kb interstitial deletion of chromosome Xp22.2, centered on the *AP1S2* gene, by means of oligonucleotide array comparative genomic hybridisation (array-CGH) in a child with marked hypotonia in the first months of life, psychomotor retardation, severely delayed walking and speech development, and unspecific dysmorphic facial features. The deletion was inherited from the healthy mother. Point mutations of the *AP1S2* gene have been identified in patients with X-linked mental retardation (XLMR). The clinical features of our patient are quite similar to those reported in male patients carrying point mutations, thus suggesting that point mutations and deletions of the *AP1S2* gene lead to a recognisable XLMR phenotype in males.

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1. Introduction

Mental retardation (MR) affects about 1–3% of the population. It is more common in males (an excess of about 30%) [1] because of the high incidence of mutations in genes located on the X chromosome that together explain 10–12% of MR in males [2]. More than 80 X-linked mental retardation (XLMR) genes have so far been identified [3,4]. XLMR has been classified into syndromic and non-syndromic forms, depending on whether the MR is associated with other clinical, radiological or metabolic features or not, but this subdivision appears unsatisfactory since many genes are involved in both forms [2,5].

The *AP1S2* gene (OMIM ID: 300629), which maps to Xp22.2, has been proposed as a candidate for XLMR because it has been found to be mutated in seven unrelated XLMR families [6–8]. It encodes the σ 1B subunit of clathrin-associated adaptor protein complex 1 (AP1), which is involved in transporting proteins between the trans-Golgi network and endosomes and interacts with synaptic vesicle proteins, thus playing an important role in neurotransmitter release [9].

We describe a child with psychomotor retardation, severely delayed walking and speech development, marked hypotonia and unspecific dysmorphic facial features, who was found to

carry a cryptic interstitial 495 Kb deletion at Xp22.2, including the *AP1S2* gene.

2. Clinical report

The patient is the only child of a healthy and unrelated 38-year-old father and 37-year-old mother, whose family histories are negative for genetic diseases. The results of prenatal ultrasonography were normal. The child was born by spontaneous vaginal delivery at a gestational age of 39 weeks; at birth, his weight was 3.450 kg (50th percentile), length 51 cm (50th percentile), occipitofrontal circumference (OFC) 34 cm (25th percentile), and APGAR scores 8 and 10 after respectively 1 and 5 min. No major medical problems were observed during the neonatal period except for poor sucking. Psychomotor delay and hypotonia became evident at the age of 4 months. The results of various clinical and instrumental examinations (echocardiography, ophthalmological screening, brain magnetic resonance imaging and computed tomography [CT], muscle CT, electroencephalography, evoked visual and acoustic potentials, electroretinography) were normal; electromyography and muscle biopsy revealed only slight and unspecific anomalies. The neurological follow-up confirmed the marked hypotonia and delayed achievement of the first psychomotor milestones (nuchal stability at 9 months, sitting alone at 12 months). The child showed also hyperreflexia, poor social interactions and stereotypies (repetitive hand movements).

He was referred to us at the age of 13 months, when his weight was 9.920 kg (10–25th percentile), height 81 cm (75th percentile)

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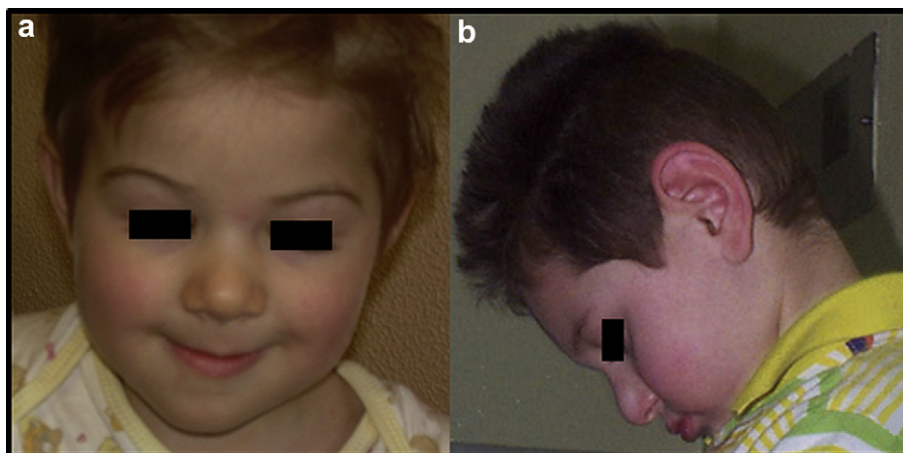


Fig. 1. Frontal photograph of the patient at the age of 13 months (a) and lateral photograph at the age of 4 years (b) showing a long face, bitemporal narrowing, and a bulbous nasal tip.

and OFC 45 cm (10th percentile). His dysmorphic facial features included a long face, bitemporal narrowing, deeply-set eyes, strabismus, up-slanting palpebral fissures, and a bulbous nasal tip (Fig. 1a).

The results of methylation test of the 15q11.2 region, study of subtelomeric rearrangements, and molecular analysis of the *UBE3A* gene were normal.

He underwent his last follow-up evaluation at the age of 4 years, when his weight was 13.5 kg (3–10th percentile), height 107.5 cm (90th percentile) and OFC 48.5 cm (3rd percentile). A clinical examination did not show any change in his facial phenotype (Fig. 1b), and he was also affected by hypermetropia and constipation. The neuropsychiatric follow-up confirmed the psychomotor delay, with significantly delayed walking and speech

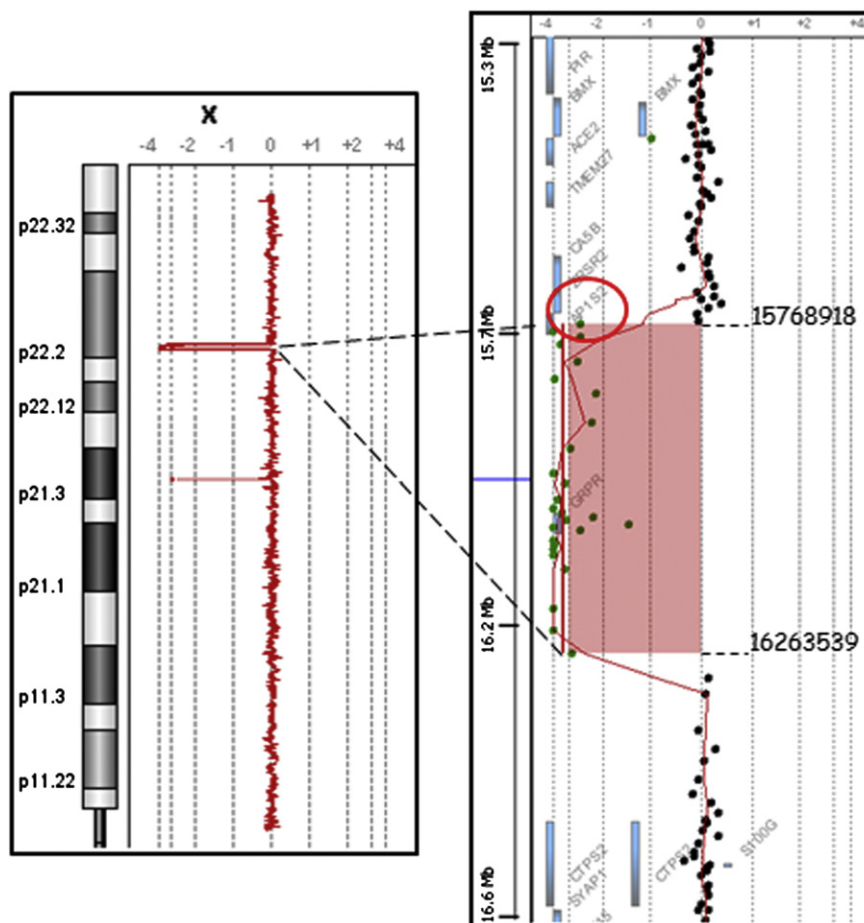


Fig. 2. Array-CGH profile. Left) Ideogram of the Xp chromosome with the log₂ probe ratio plotted as a function of chromosomal position. Right) Magnification of the deleted region indicating the distal and proximal breakpoint positions and the included genes.

development, and revealed a slow improvement in the hypotonia and social interactiveness.

3. Material and methods

Array-CGH of genomic DNA from the proband and his parents was performed using the Human Genome CGH Microarray kit 44K (Agilent, Santa Clara, CA) and, restricted to the proband, the Human Genome CGH Microarray kit 244A (Agilent). These platforms allow a genome-wide survey with an average resolution of respectively ~130 and ~50 Kb. The genomic DNAs were labelled and hybridised in accordance with the manufacturer's protocol, and the arrays were analysed using Agilent Scan Control (v A8.4.1), Feature Extraction software (v 10.7.1.1), and DNA Analytics software (v 4.0). The unbalanced region revealed by the analysis was analysed *in silico* using the March 2006 release of the UCSC Genome Browser (<http://genome.ucsc.edu/>) and the Database of Genomic Variants (<http://projects.tcag.ca/variation>).

The methylation status of the X chromosome in the patient's mother was assessed using the Humara Androgen Receptor gene methylation assay as previously described [10]. The triplet repeats were separated by means of capillary electrophoresis and analysed using an ABI PRISM 3005.

4. Results

Array-CGH using the Human Genome CGH Microarray kit 44K revealed the presence of an interstitial Xp22.2 deletion (not shown), which was confirmed and more precisely defined by means of the higher-resolution 244K platform. The breakpoints of the ~495 Kb deletion are located proximally between 16,263,539 and 16,300,564 bp and distally between 15,763,401 and 15,768,918 bp (Fig. 2). The microdeletion includes only two genes: *AP1S2* and *GRPR* (Fig. 2).

The deletion was not detectable by array-CGH in the father. The healthy mother appeared carrier of the same imbalance as the child, while no skewed X-inactivation could be detected (data not shown).

5. Discussion

Only point mutations in the *AP1S2* gene have so far been reported [6–8]. Altogether, three different nonsense mutations (c.154C > T, c.106C > T and c.226G > T) and three splice site mutations (c.180-5_180-1del4, c.288+5G > A and c.289-1G > C) have been identified in seven families [8], including three that have allowed the detection of a linkage to the *AP1S2* region [11–14].

All of the previously described affected males have varying degrees of intellectual disability (from mild to profound) and their early lives were characterised by marked hypotonia; furthermore, most of them experienced delayed walking and speech development, language disturbances (from no language to minimal language), without any significant dysmorphic features. The patients for whom a radiological description is available also showed calcifications of the basal ganglia (see Borck et al. [8] for a detailed summary of the clinical features of patients harbouring *AP1S2* mutations). The identified *AP1S2* mutations co-segregating with the disease in the described families seem to underlie a specific and recognisable XLMR syndrome.

The clinical findings in our patient reflect most of those observed in the carriers of deleterious mutations: psychomotor retardation, a significant delay in walking and speech development, marked hypotonia in the first months of life that subsequently slightly improved, and unspecific dysmorphic facial features. A CT scan at the age of 4 months excluded the presence of cerebral

calcifications, which is consistent with their reported delayed appearance in childhood [8].

As previously observed in unaffected female mutation carriers, the proband's mother, who is heterozygous for the *AP1S2* deletion, shows random X-inactivation and a normal phenotype, thus indicating that gene haploinsufficiency is insufficient to induce the pathological clinical features.

The *GRPR* gene (OMIM ID: 305670) close to *AP1S2* is also deleted in the child (Fig. 2). This gene encodes the gastrin-releasing peptide receptor (GRPR), which regulates a broad spectrum of biological responses, acts as a growth factor in different types of human cancer, and has been implicated in the pathogenesis of mammalian neurological and psychiatric disorders, including the neurodevelopmental disorders associated with autism [15,16].

In his early infancy, our patient showed hand stereotypies and poor social interactions, which are usually reported in patients with autistic spectrum disorders (ASD). However, it is hazardous to consider a correlation with the absence of this gene as the findings of a recent study of 149 Italian autistic patients do not indicate that *GRPR* plays a major role in ASD [17]. The stereotypies of our patient may therefore also be associated with the *AP1S2* deletion because two of the described XLMR patients with stereotypies have been diagnosed as having ASD [8].

In conclusion, ours is the first description of a patient with syndromic MR and an *AP1S2* deletion. The absence of *AP1S2* leads to clinical features that overlap those associated with inactivating point mutations in males, thus confirming the involvement of this gene in a recognisable XLMR syndrome.

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