Original Article

Journal of Child Neurology
Volume XX Number X
Month XXXX xx-xx

10.1177/0883073808316367
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Angelman Syndrome Due to a Novel Splicing Mutation of the *UBE3A* Gene

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Angelman syndrome is a neurodevelopmental disorder characterized by mental retardation, absence of speech, seizures, abnormal electroencephalography (EEG), and happy disposition. The syndrome results from lack of function of the maternal copy of the *UBE3A* gene on the imprinted Prader Willi/Angelman syndrome critical region; it is caused by large deletions, paternal uniparental disomy, imprinting center defects or *UBE3A* deletions, and point mutations. We found a novel splice-site mutation of the *UBE3A* gene in a child with clinical and EEG features of Angelman syndrome. This case further points out the fact that individuals with Angelman syndrome and mutations of the *UBE3A* gene have a phenotype that tends to be rather mild, however, undistinguishable, both from the clinical and the electrophysiological points of view from the Angelman syndrome phenotype due to other known molecular mechanisms.

Keywords: Angelman syndrome; mutation; phenotype; splice-site; *UBE3A*

Angelman syndrome [MIM 105830] is a neurodevelopmental disorder characterized by profound cognitive and motor delay, severe speech impairment, epilepsy with characteristic electroencephalography (EEG) abnormalities, subtle dysmorphic facial features, and happy disposition with a tendency to frequent and inappropriate laughter. The other main manifestations of the syndrome are ataxic gait, jerky movements, myoclonus of cortical origin, hyperexcitable personality, postnatal onset microbrachycephaly, and sleep disorders.[1-4] This syndrome results from lack of function of the maternally inherited copy of the Ubiquitin-Protein Ligase E3A (*UBE3A*) gene located at 15q11.2, in the imprinted Prader-Willi/Angelman syndrome critical region. Multiple mechanisms can disrupt the function of the maternal copy of *UBE3A*. In the majority of cases, this disruption occurs by 1 of the 4 following different mechanisms: large interstitial deletions (70%), paternal uniparental disomy (3–5%), imprinting center defects (2–6%), and intragenic *UBE3A* mutations (4–23%).[5-7] We report and discuss, in light of the available literature, a case of Angelman syndrome due to a novel splicing mutation of the *UBE3A* gene.

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Sartori S, Anesi L, Polli R, Toldo I, Casarin A, Drigo P, Murgia A. Angelman syndrome due to a novel splicing mutation of the *UBE3A* gene. *J Child Neurol.* 2008;XX:XXX-XXX.

Case Report

The girl we describe is the second child of healthy and nonconsanguineous parents. The family history is negative for hereditary disorders, and the pregnancy was unremarkable, without exposures to known teratogens. Prenatal ultrasounds were normal, and the child was born at term by spontaneous and vaginal delivery. At birth, the weight of the child was 2.8 kg (10th centile), the length 48 cm (10th–25th centiles) and the head circumference was 33.5 cm (25th–50th centiles). In the first year of life, she showed a mild delay of developmental milestones: head control at 3 months; sitting at 7 months, rolling at 12 months, aided walking at 14 months. She never walked without support and never reached the ability of speaking; her head growth started to slowly decelerate from the first months of age. She was described by the parents as a quiet baby with a happy disposition; no regression of acquired skills was reported. She never had seizures.

At the time of evaluation, the child was 16 months old, her height was 78.5 cm (50th percentile), the weight 8.9 kg (3rd–10th percentile), and the occipitofrontal circumference 43.5 cm (3rd percentile). She had mild microcephaly, mild mid-face hypoplasia, a broad nasal bridge, anteverted nares, large and prominent ears, strabismus, short and smooth philtrum, wide mouth, small and wide-spaced

Figure 1. The patient at 16 months of age.

teeth, a high palate and she was frequently drooling (Figure 1). Her fair complexion, with blond hair and blue eyes, was consistent with the other members of the family. At the age of 16 months, she was able to sit and pull-to-stand independently, but she could walk only a few unsteady steps with support. She could move independently by wriggling forward on her buttocks (bottom shuffling). Speech was completely absent, but her receptive and nonverbal communication skills were fairly conserved. On examination, she showed a very happy and sociable disposition, she smiled when given attention but she often had bursts of inappropriate laughter. She observed and played with the objects around her with inconstant interest. At the age of 16 months, her first EEG showed, in the awake state, generalized high voltage slow waves (exceeding 200 μV), configuring prolonged runs of rhythmic 4–5/s θ activity, sometimes with centrotemporal and occipital predominance. This activity, scarcely modulated by eye closure, was similar to the θ pattern reported by Dan and Boyd.[8] Magnetic resonance imaging (MRI) of the brain did not detect any structural abnormality.

Currently, at 30 months of age, the child can walk without support, and she climbs up and down the stairs autonomously; she says a few intelligible words that are usually appropriate to the context and is beginning to communicate by means of a strategy of augmentative-alternative communication.

Molecular Methods

Regular informed consent for genetic testing on the child was obtained before the analysis. Genomic DNA was extracted from peripheral blood leukocytes with standard methods. Methylation analysis of the Prader-Willi/Angelman critical region of chromosome 15 was performed by methylation-specific polymerase chain reaction (PCR), according to Kosaki et al.[9] Briefly, genomic DNA was modified with sodium metabisulfite, and exon 1 of the small nuclear ribonucleoprotein-associated polypeptide N(*SNRPN*) gene was PCR-amplified with allele-specific primers. Amplification products were electrophoresed on a 10% polyacrylamide gel and visualized by silver staining.

Mutation scanning of the *UBE3A* gene was performed by PCR amplification and direct sequencing of fragments representing all the coding exons and intron–exon boundaries, with primers and conditions slightly modified by Rapakko et al.[10] Novel primers were designed for exon 9 to exclude the risk of coamplifying the processed *UBE3A* pseudogene (*UBE3AP2*; primers sequence and amplification conditions available upon request). Direct sequencing of the amplification products was performed, using the Big-Dye Terminator ready reaction kit v.3.1 (Applied Biosystems, Warrington, UK) on an ABI 3100 automated DNA sequencer (Applied Biosystems). The obtained sequences were compared with the reference *UBE3A* sequence (GenBank NG_002690) by BLAST analyses.

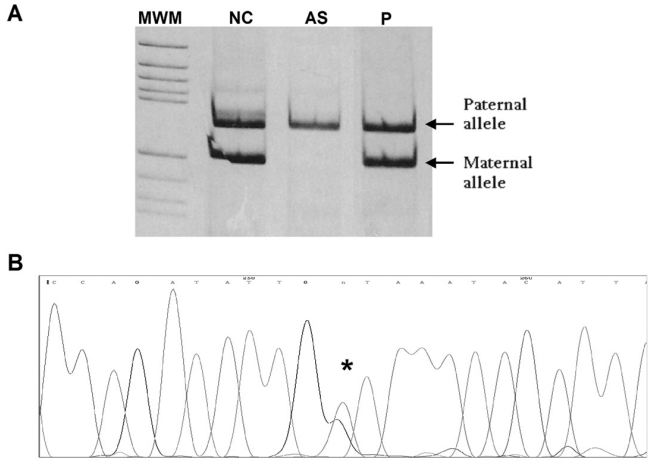

Figure 2. A, Polyacrylamide gel electrophoresis of methylation-specific polymerase chain reaction products of exon 1 of the *SNRPN* gene. Lane 1, Molecular weight marker (MWM; PBR322 by *Hae*III). Lane 2, Normal control (NC) showing a normal methylation pattern with both maternal and paternal contribution. Lane 3, Angelman syndrome control (AS) displaying an abnormal methylation pattern with absence of the maternal contribution. Lane 4, Proband (P) sample showing a normal methylation pattern. B, Direct sequencing of the *UBE3A* amplicon harboring a heterozygous G to A substitution (asterisk).Establishing the parental origin of the mutated *UBE3A* allele was not possible due lack of informativity of the family for the analyzed single nucleotide polymorphisms.

The segregation of the *UBE3A* mutated allele was studied in the proband and in the parents by PCR amplification and subsequent sequencing of a region of approximately 2 kb encompassing the mutation and including 9 single nucleotide polymorphisms (rs2719881, rs17115502, rs28675328, rs10713541, rs36024661, rs10162823, s3043907, rs34477605, rs11856839); primers used are as indicated in the Ensemble Genome Browser database.

Results

The methylation-specific PCR analysis performed on the DNA of the proband evidenced the presence of a correctly imprinted biparental contribution at the Prader-Willi/Angelman critical region (Figure 2A). Mutation scanning of the *UBE3A* coding exons and flanking intronic regions allowed detection of the presence of a heterozygous transition replacing guanine with adenine at the first position of intron 10, immediately after position 1693 (cDNA seq. GenBank X98032) the last nucleotide of exon 10 (c.1693+1 G>A; Figure 2, B). The identified mutation, never previously reported, alters the consensus sequence of the *UBE3A* intron 10 donor splice site; the alteration was not found on the DNA of either parent of the probands.

Discussion

Our patient displays a clinical and neurophysiological picture similar to that observed in the other reported Angelman syndrome patients with *UBE3A* mutations. The severity of the phenotype in these individuals tends to fall in the middle between what is seen in Angelman syndrome due to large deletions and to uniparental disomy. Individuals with *UBE3A* mutations are in fact similar to deletion patients with respect to seizures, absent speech, and microcephaly, whereas they are closer in their better motor skills, ability to follow simple commands, and relatively spared communication skills, to uniparental disomy patients.[3,5,11] That the Angelman syndrome clinical profile is more severe in individuals carrying *UBE3A* mutations than in patients with uniparental disomy may be interpreted as a further indication of the central role played by this gene in the pathogenesis of the syndrome.

In our patient, the molecular analysis revealed a de novo splicing mutation of the *UBE3A* gene, never previously reported in the literature. This mutation alters the consensus sequence of the donor splice site of intron 10 and is likely to create a frame-shift, which in turn may result in a premature stop codon and a truncated nonfunctional protein. This finding is, therefore, a pathogenetic mutation that is to be considered responsible of the Angelman syndrome phenotype observed in the proband.

Although not demonstrable, due to lack of informativity of the polymorphic markers that could be shown to cosegregate with the mutation, we expect this de novo mutation to reside on the maternal chromosome, because the Angelman syndrome phenotype correlates with loss of maternal-specific expression of *UBE3A* in the brain.[12] A de novo mutation arising on the paternal chromosome would lead to a normal phenotype.[13]

The mutation we report is 1 of the few *UBE3A* splicing mutations described in the literature.[14,15] Indeed, to the best of our knowledge, 53 *UBE3A* disease-causing mutations have so far been found in patients with Angelman syndrome: 13 missense/nonsense mutations, 21 small deletions, 15 small insertions, 1 gross deletion, 1 complex rearrangements and only 2 splicing mutations (www.hgmd.cf.ac.uk).

Although mutations have been found in virtually all protein-coding exons, most of them cluster within exons 9 or 16 of the *UBE3A* gene and are predicted to give rise to truncated E6-AP ubiquitin–protein ligase.[11,16] The majority of *UBE3A* mutations appear to occur de novo, and only approximately 20% of the mothers carry the same mutation as their affected children. There are, however, several reports of mothers, likely gonadal mosaics, who despite a negative *UBE3A* analysis have more then 1 affected child. For this reason, it is advisable that all

mothers of children with *UBE3A* mutations, even though apparently de novo, be offered prenatal testing in future pregnancies.[3]

Our data confirm that, although the overall phenotype of patients with *UBE3A* defects seems milder than what is observed in Angelman syndrome patients with large deletions, nevertheless their clinical picture is fairly classic. All the known genetic mechanisms causing Angelman syndrome lead to a phenotype largely characterized by severe-to-profound mental retardation, movement disorders, myoclonus, characteristic behavior, and severe limitations in speech and language. Despite subtle clinical differences—that somehow correlate with the distinct molecular categories—individuals with Angelman syndrome are more alike in their clinical features than they are different.[5] In other words, the different genetic defects responsible of this syndrome are not actually recognizable on the basis of a mere clinical and neurophysiological evaluation. The few published cases of Angelman syndrome with alterations of the *UBE3A* gene, including our report, do not allow delineation of specific genotype–phenotype correlations.[17,18]

Methylation analysis of the Prader-Willi/Angelman S critical region remains the first diagnostic step in children with psychomotor delay, happy disposition, hyperexcitable personality, characteristic EEG abnormalities, mental retardation, and severe speech impairment. When this genetic test evidences the presence of a correctly imprinted biparental contribution, then a complete molecular analysis of the *UBE3A* gene is mandatory. A molecularly confirmed diagnosis of Angelman syndrome, which avoids further unnecessary diagnostic procedures, is crucial for an appropriate counseling and for prenatal diagnosis.

Further reports of *UBE3A* mutations in Angelman syndrome individuals will contribute to a better characterization of the molecular pathology of this gene and possibly to establishing genotype–phenotype correlations.

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