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Discordant Phenotypes in First Cousins With *UBE3A* Frameshift Mutation

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Mutations have been found in the UBE3A gene (E6-AP ubiquitin protein ligase gene) in many Angelman syndrome (AS) patients with no deletion, no uniparental disomy, and no imprinting defect. UBE3A mutations are more frequent in familial than in sporadic patients and the mutations described so far seem to cause similar phenotypes in the familial affected cases. Here we describe two first cousins who have inherited the same UBE3A frameshift mutation (duplication of GAGG in exon 10) from their asymptomatic mothers but present discordant phenotypes. The proband shows typical AS features. Her affected cousin shows a more severe phenotype, with asymmetric spasticity that led originally to a diagnosis of cerebral palsy. Proband's brain MRI shows mild cerebral atrophy while her cousin's brain MRI shows severe brain malformation. This family demonstrates that, although brain malformation is unusual in AS, presence of a brain malformation does not exclude the diagnosis of AS. Also, this UBE3A mutation was transmitted from the cousin's grandfather to only two sisters among eight full siblings, raising the hypothesis of mosaicism for this mutation.
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KEY WORDS: Angelman syndrome; *UBE3A* gene; mutation screening

INTRODUCTION

In 1965, Harry Angelman [Angelman, 1965] described three unrelated children with similar clinical characteristics of mental retardation, flat heads, seizures, spastic movements, protruding tongue, absent speech, paroxysms of laughter, and ataxic gait—a condition now called Angelman syndrome (AS).

AS is clinically characterized by central congenital hypotonia, delayed neuropsychomotor development, severe mental retardation, total or almost total lack of speech, excessive laughter, hyperactivity, and dysmorphic features such as micro-brachycephaly, macrostomia, widely-spaced teeth, lingual protrusion, mandibular prognathism [Clayton-Smith and Pembrey, 1992]. And, neurologically, the patients have seizures, ataxic movements, and characteristic EEG findings [Boyd et al., 1988].

There are different genetic mechanisms leading to the occurrence of AS. Most AS cases (70%) are caused by a de novo deletion in the 15q11-13 region of the maternally inherited chromosome 15 [Knoll et al., 1989]. This group also includes cases caused by chromosomal rearrangements leading to microdeletions in this region. A small percentage (3–5%) of patients with AS shows paternal uniparental disomy of chromosome 15 [Malcolm et al., 1991]. Defective imprinting of the 15q11-13 region accounts for 7–9% of cases [Buiting et al., 1995]. In 4–8% of cases, the affected individuals show mutations in the *UBE3A* gene [Kishino et al., 1997; Matsuura et al., 1997] and in 10–15% of cases the molecular exam is normal with no deletion, uniparental disomy, imprinting defect, and *UBE3A* mutation [Fang et al., 1999].

All known causes of AS involve lack of a functioning maternal copy of the *UBE3A* gene, which encodes the E6-AP ubiquitin-protein ligase. *UBE3A* is subject to tissue-specific imprinting, since in brain tissue the maternal allele is expressed at much higher level than the paternal allele [Albrecht et al., 1997; Rougeulle et al., 1997; Vu and Hoffman, 1997]. The *UBE3A* gene includes at least 16 exons that span approximately 100 kb and has a mRNA size of 5–8 kb, and undergoes alternative splicing to produce five different types of mRNA [Yamamoto et al., 1997; Kishino and Wagstaff, 1998]. E6-AP is responsible for defining the substrate specificity for ubiquitin transfer and for directly catalyzing ubiquitin transfer to substrates [Scheffner et al., 1995].

We have studied 96 patients with a clinical suspicion of AS in whom no deletions, no uniparental disomy, and no imprinting mutations were present. We have found two affected first cousins who have inherited the same UBE3A frameshift mutation from their asymptomatic mothers, but show discordant phenotypes.

PATIENTS AND METHODS

Patients

Between 1995 and 2001, 96 patients with the clinical diagnosis of AS (87 sporadic and 9 familial patients), showing normal SNRPN methylation pattern, were seen at or referred to the Medical Genetics Unit of the University Hospital of the School of Medicine of Ribeirao Preto. The clinical diagnosis of AS was made based on criteria described by Williams et al. [1995]. Deletions, uniparental disomy, and imprinting defects were excluded in all these patients by a combination of FISH analysis, methylation analysis, and polymorphism analysis. Informed consent for the study was obtained from all families.

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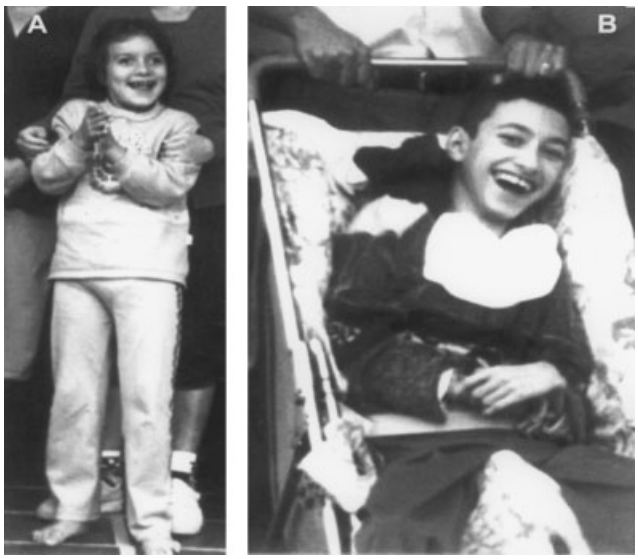

Fig. 1. First cousins with *UBE3A* mutation. **A**: Patient with typical AS phenotype (III.1); (**B**) patient with severe phenotype (III.14).

The two patients (III.1, III.14) (Fig. 1) give similar results in cytogenetic and molecular analysis but their phenotype is quite different. III.1, the proband, aged 9 years, was born to non-consanguineous and healthy parents. After a normal pregnancy, the patient was born at term by normal delivery, weighing 3,000 g (25–50th centile) and measuring 47 cm (25th centile). Parents reported sleep disorders since 1 month of age. Her developmental progress was delayed. She sat at 3 years old and walked at 3 years and 6 months, and she is not able to speak. At age of 5 years old, she began to have seizures and has been on carbamazepine with good control. She has normal EEG and brain MRI showing mild cerebral atrophy (Fig. 2). When she was examined at the age of 5 years and 11 months, her head circumference was 51 cm (25th centile), height 115 cm (25–50th centile), and weight 2,500 g (97th centile).

III.14, the proband's cousin, aged 15 years and 9 months, was born to a non-consanguineous and healthy parents. The prenatal period was uneventful except for the fact that it was a twin gestation. At delivery, III.14 was born together with a deceased co-twin. His parents do not know if the deceased child was male or female and they do not have any further information about the causes of the baby's death. III.14 was delivered by cesarean section at full term with a birth weight of 3,200 g (50th centile), height of 48 cm (25–50th centile), and head circumference of 32.5 cm (10–25th centile). His developmental progress was delayed. He presented with hypotonia by age of 3 months old, but at the age of 6 months, he was given the diagnosis of cerebral palsy due to clinical presentation of hypertonicity of four limbs and trunk hypotonia. At the present moment, he is not able to walk, has spasticity of the limbs and a significant scoliosis of the spine, and is maintained in a wheelchair, except for sleep. He is not able to speak, and has sleep disorder. At 1 year of age, he had the first episode of seizures and, at the age of 9 years he started with seizures. He has been treated with valproic acid and carbamazepine with good control. He has normal EEG. His brain MRI shows dysplastic cortex with irregular, bumpy outer and inner surface and irregular gray-white matter junction around the Sylvian fissures, bilaterally, with a quite symmetrical pattern. On the posterior frontal and anterior parietal lobes, the cortex folds inwards with a profound sulcus surrounded by the same pattern of dysplastic cortex. The bilateral infoldings resemble a closed lip schizencephalic cleft, but they do not reach the ventricular surface which shows no sign of any dimple in it walls. There is a clear portion of normal white matter between the microgyric cortex infolded and the ventricular border. The cortical appearance with isointense sign and bilateral symmetric opercular region involvement allows the radiological diagnosis of a congenital bilateral perisylvian polymicrogyria (Fig. 3).

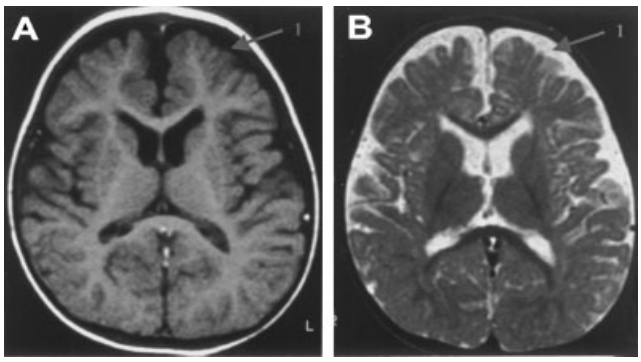

Fig. 2. Magnetic resonance images from the proband. **A**: T1-weigheted image. **B**: T2-weigheted image. Both are axial slices from Spin Echo sequences. The enlarged CSF space and rounded ventricle suggest mild atrophy (arrow 1).

Mutation Detection

Genomic DNA was extracted from peripheral blood by standard methods. We used the SSCP technique to screen the *UBE3A* gene for mutations. For the SSCP-PCR, we amplified all the sixteen exons of *UBE3A* gene based on Malzac et al. [1998]. When an abnormal shift was found, we cut out the normal as well as the mutant shifts, eluted them in water, and reamplified by PCR. The final PCR product was purified and sequenced on an ABI 377® automated sequencer and the sequences were compared with GenBank accession no. U84404, according to Kishino and Wagstaff [1998]. To confirm the mutation, the SSCP-PCR and the DNA sequencing were

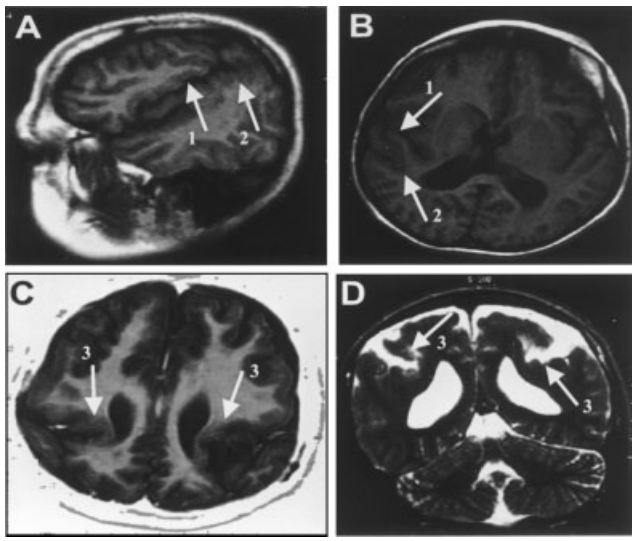

Fig. 3. Magnetic resonance images from the proband's cousin. **A**: Spin-Echo T1-w sagittal sequence. **B**: Spin-Echo T1-w axial sequence. **C**: Inversion recovery T2-w axial sequence with video-inversion. **D**: Spin-Echo T2-w coronal sequence. Note the irregular cortex with bumping surface (arrow 2) around the lateral fissure (arrow 1) bilaterally, with parietal infolding without ependymal involvement (arrow 3). The image suggests polymicrogyria.

repeated twice with both forward and reverse primers. Following confirmation of each mutation, we screened for the same mutation in 50 unrelated normal controls (100 alleles).

Linkage Analysis

Haplotyping analysis was performed using primers for three microsatellite markers in the 15q11-13 region (D15S11, D15S122, and GABRB3). The primers sequences and PCR conditions were as indicated in the Genome Data Base.

RESULTS

The two first cousins have inherited the same *UBE3A* frameshift mutation from their asymptomatic mothers but who show discordant phenotypes (Fig. 1, Table I). The mutation is a novel duplication of GAGG in exon 10. The proband shows typical AS features and typical AS developmental history. Her affected cousin shows a more severe phenotype, with hypertonicity of four limbs and trunk hypotonia that led originally to a diagnosis of cerebral palsy. The proband's brain MRI (Fig. 2) shows mild cerebral atrophy while her cousin's brain MRI (Fig. 3) shows a more severe brain abnormality, suggesting polymicrogyria.

Linkage analysis (Fig. 4) shows that the grandfather must have been a mosaic for the *UBE3A* mutation: the same haplotype (3-1-3) contains a *UBE3A* mutation in II.2 and II.14, but contains no *UBE3A* mutation in II.3 and II.5.

DISCUSSION

This family with *UBE3A* mutation shows discordant AS phenotypes in two cousins with maternal inheritance of the same *UBE3A* mutation; a frameshift mutation, caused by duplication of GAGG in exon 10, that creates a premature stop codon leading to a truncated protein [Molfetta et al., 2003].

TABLE I. Clinical Findings of the Patients With AS (Based on Williams et al., 1995)

Clinical characteristics	III.1	III.14
Consistent (100%)		
Developmental delay, functionally severe	+	+
Speech impairment	+	+
Movement or balance disorder, usually ataxia of gait	−	NE
Happy behavior	+	+
Frequent (more than 80%)		
Delayed growth in head circumference	+	+
Seizures	+	+
Abnormal EEG	−	+
Associated (20–80%)		
Flat occiput	+	+
Occipital groove	−	−
Protuding tongue	+	−
Tongue thrusting; suck/swallowing disorders	−	+
Feeding problems during infancy	+	+
Prognathism	−	+
Wide mouth, wide-spaced teeth	+	+
Frequent drooling	+	+
Excessive chewing/mouthing behaviors	−	−
Strabismus	+	+
Hypopigmented skin	−	−
Hyperative lower limb deep tendon reflexes	−	−
Uplifted, flexed arm position	+	−
Increased sensitivity to heat	+	−
Sleep disturbance	+	−
Attraction to/fascination with water	+	−

NE, not examined.

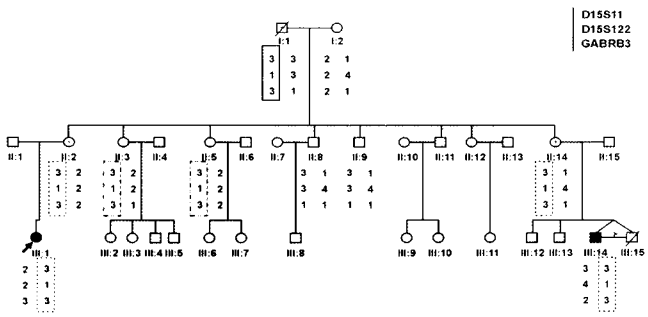

Fig. 4. Pedigree, microsatellite analysis and haplotyping. Segregation of the normal (dashed line) and mutant (dotted line) chromosomes; solid line represents the mosaic chromosome. The grandparents' genotype were inferred while individuals who do not show a genotype are those who did not agree to participate in this study.

This protein is likely to be inactive because a UBE3A mutant protein lacking the last six amino acids is completely defective in ubiquitination [Huibregtse et al., 1995].

The family described here is the first example of a family with two first cousins sharing the same *UBE3A* mutation and showing discordant phenotypes. Previously described familial cases of *UBE3A* have shown similar phenotypes in affected family members [Matsuura et al., 1997; Fung et al., 1998; Moncla et al., 1999; Russo et al., 2000].

This family also demonstrates that the presence of a brain malformation does not exclude the diagnosis of AS. In spite of the severe brain malformation, the proband's cousin shows better cognitive performance and better interaction with the environment than the proband.

We have three hypotheses to explain the phenotypic discordance between the cousins: (1) the second cousin has an additional problem (genetic or environmental) besides the *UBE3A* mutation that has caused the brain malformation; (2) the *UBE3A* mutation is interacting with a different genetic variant in the second cousin that, by itself, does not cause problems but in combination with the *UBE3A* mutation causes the very severe phenotype; or (3) this *UBE3A* mutation alone can cause either typical AS or the very severe clinical picture seen in the second cousin.

Bilateral perisylvian polymicrogyria and schizencephaly are cortical malformations with similar MRI appearance classified as malformations due to abnormal cortical organization. They are frequently observed together in patients and can be seen in different members of the same family [Barkovich et al., 2001]. Yakovlev and Wadsworth described schizencephaly in 1946 as a malformation with clefts within the cerebral hemispheres with cortical gray matter lining their borders with a fusion of the pial surface and the ventricular ependyma forming a pial-ependymal seam. The lips can be closed or opened but the sign of ventricular involvement with the cleft is a hallmark of the malformation, sometimes reduced to a not obvious nipple, but always present because the pathogenesis of this anomaly is a segmental failure in the formation of a portion of the germinal matrix with a whole band of absent parenchyma. On the other hand, the bilateral perisylvian polymicrogyria is a post migrational defect where the neurons reach the cortex but are totally infunctional or lack the normal cortical six-layered organization with formation of an unlayered or a four-layered cortex, disorganized, with multiple small gyros and bumping surface, sometimes with infolding which can be followed by anomalous vessels, but not related to ventricular surface [Foix et al., 1926]. The MRI of our patient shows bilateral clefts arising from the Sylvian fissures that do not reach the ventricules, as well as areas of polymicrogyria surrounding the Sylvian fissures. Because the clinical presentation is not

standard, the presence of polymicrogyria in the MRI has been an important diagnostic criteria.

Considering the fact that this was a twin pregnancy and that the second baby did not survive the prenatal period, it is possible that III.14's brain malformation might be due to a vascular event caused by in utero death of the twin; it is also possible that the cause of the death of the twin may be the cause of the brain malformation in III.14; thirdly, there is a possibility that both babies presented the same spectrum of abnormalities and that these abnormalities led to in utero death in one twin but not in the other twin. Barth [1987] described an instructive case where one twin died in utero and the other presented unlayered polymicrogyria in a vascular distribution after intrauterine infection.

Monozygotic twinning has been associated with a variety of vascular disruptive events [Jung et al., 1984; Patten et al., 1989; Van Bogaert et al., 1998]. The mechanism resulting in the lesions may have been a transient cerebral vascular compromise associated with placenta vascular anastomoses characteristic of monochorionic twinning [Perlman et al., 1995]. The classical situation is when the recipient twin was affected and his co-twin, the donor was macerated. Lesions in the recipient twin may result from emboli or thromboplastic material originating from the macerated twin. Blood pressure instability or episodes of severe hypotension might lead to brain and/or visceral lesions in the recipient twin. In the donor, the lesions result from hypotension and/or anemia [Larroche et al., 1990]. These abnormalities lead to the development of brain lesions, such as encephalomacia (when brain is affected later in pregnancy) or dysgenesis, manifested as microgyria and heterotopias (when development is disrupted at early stages of morphogenesis) [Scheller and Nelson, 1992].

It is controversial whether polymicrogyria is only a destructive process or has a malformative component. Some authors have described familial recurrence of bilateral perisylvian polymicrogyria with genetically heterogeneous pattern with some families with X-linked transmission [Guerreiro et al., 2000]. The four-layered variant is most frequently considered to result from a destructive lesion, which occurs at approximately 20–24-week gestation and the unlayered form is thought to result from an earlier insult around 13–16 weeks [Mischel et al., 1995]. Many experimental models, like coagulation lesions in newborns rats, suggest an in utero circulatory disorder as a principal cause of polymicrogyria, which is supported by fetal pathology observations of four-layered polymicrogyria in carbon monoxide accidents in pregnant mothers at 20–24 weeks.

Another unusual observation regarding this *UBE3A* mutation is the fact that it was transmitted from the cousin's grandfather to only two sisters among eight full siblings. As the expected rate of normal carriers within the sibship would be 50% if the maternal grandfather were a normal carrier, we hypothesized that the transmitting grandfather, now deceased, may have been mosaic for this mutation. We have shown that the same 15q11-13 haplotype that carries the *UBE3A* mutation in the mothers of the affected cousins carries no *UBE3A* mutation in two of their sisters, thereby confirming mosaicism which must have been in their father, because the mothers of the affected twins are phenotypically normal and, it is extremely unlikely that two identical, new spontaneous mutations would occur in the same kindred. Malzac et al. [1998] showed that 3 out of 13 newly arising *UBE3A* mutations arose in mosaic individuals, and our findings are consistent with their observation.

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