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 [Butting 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; Rougelle 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 Ribeirão 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.
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. 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).

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

NE, not examined.
standard, the presence of polymicrogyria in the MRI has been an important diagnostic criterion.

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 encephalomalacia (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 [Guerrero 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.

ACKNOWLEDGMENTS

The authors are grateful to the families who were willing to participate in this study. They are also grateful to the clinicians who referred patients for their cooperation in this study, especially Dr. Temis Maria Felix and Dr. Vera Gil Silva Lopes. They further thank Dr. Victor E.F. Ferraz for all his help.

REFERENCES


