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Clinical Report

Atypical Cases of Angelman Syndrome

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Angelman syndrome (AS) is a profound disorder notable for mental retardation and severe language deficits that results from lack of function of the maternally inherited copy of the *UBE3A* gene. Chromosome deletions of 15q11q13, paternal uniparental disomy (UPD), *UBE3A* gene mutations, and imprinting center defects are all commonly recognized mechanisms that disrupt the function of the maternal copy of the *UBE3A* gene. We report here two patients with different atypical etiologies of AS. The first patient is a 3-year-old boy with global developmental delay, severe speech deficits, seizures, and very happy disposition. Southern blot analysis for the maternal and paternal chromosome 15 methylation products showed a mosaic methylation pattern, suggesting an imprinting center defect. The second patient is a 4½-year-old boy with global developmental delay, no expressive language, microcephaly, seizures, and ataxic gait. Array-based comparative genomic hybridization (CGH) demonstrated a loss in copy number for two overlapping clones encompassing the *UBE3A* gene, indicating a partial deletion within *UBE3A*. His mother, who was adopted, had an identical pattern, suggesting that her deletion was probably on her paternally imprinted allele. These patients illustrate the expanding spectrum of molecular findings in AS, reinforce the need to maintain suspicion when clinical features suggest AS but initial testing is normal, and show the power of CGH as a tool to uncover partial *UBE3A* deletions.

Key words: Angelman syndrome; comparative genomic hybridization; UBE3A

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INTRODUCTION

Described by Harry Angelman in 1965, Angelman syndrome (AS) is a neurodevelopmental disorder characterized by profound speech deficits, gait ataxia, seizures, characteristic EEG, microcephaly, and an unusually happy demeanor with propensity to paroxysms of laughter [Angelman, 1965; Boyd et al., 1988; Williams et al., 1995; Clayton-Smith and Laan, 2003]. The characteristic facial appearance includes a wide, smiling mouth with small, widely spaced teeth. AS is of particular interest as it is an imprinting disorder, in which maternal and paternal copies of genes have differential function. The underlying molecular deficit in AS is the lack of function of the maternal copy of the gene *UBE3A* (ubiquitin E3 ligase) on chromosome 15. The paternally inherited copy is normally imprinted, preventing gene expression. Thus, function of the maternal copy is essential. Disruption of paternally inherited genes within the same region give rise to Prader–Willi Syndrome, with a very different phenotype characterized by hypotonia, initial growth failure followed by obesity, hypogonadism, and variable degrees of mental retardation. Multiple mechanisms can disrupt the function of the maternal copy of *UBE3A*, including deletions at 15q11q13 of the maternally inherited chromosome [Magenis et al., 1987; Knoll et al., 1989], paternal uniparental disomy [Nicholls et al., 1992], point mutations within the maternally inherited copy of UBE3A [Kishino et al., 1997], and imprinting center defects [Buiting et al., 1995]. However, a small group remains with normal findings on this extensive panel but who has clinical features of AS, raising the question of what alternative mechanisms exist.

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Testing typically starts with methylation pattern analysis. An abnormal pattern can result from 15q11q13 deletion, UPD, or an imprinting center defect. If the methylation pattern is normal, *UBE3A* gene sequencing for mutations is performed. Although current sequencing techniques can detect most point mutations, it can fail to detect some deletions within *UBE3A*. As more children with suspected AS are tested, however, additional pathogenic mechanisms are being detected. Recent reports have revealed some cases of children with AS with somatic mosaicism for abnormal methylation patterns [Buiting et al., 2003; Nazlican et al., 2004]. Additionally, report has been made of a family with multiple affected members due to a very small interstitial deletion just encompassing the *UBE3A* gene region [Burger et al., 2002]. We report here on two patients with atypical molecular causes of AS: mosaic abnormal methylation and a novel partial microdeletion within *UBE3A*.

PATIENT 1

Patient 1 was delivered vaginally at term to his 36-year-old mother after an uneventful pregnancy without exposures to known teratogens. Prenatal ultrasounds and an amniocentesis for advanced maternal age were normal. Birth weight was 3.9 kg (between the 50th and 75th centiles) and length was 50 cm (50th centile). His head circumference at birth is unavailable. His teeth were slightly widely spaced. His demeanor was generally happy. His complexion was fair and his hair was brown, consistent with the complexion and hair of other family members. At age 3 years (Fig. 1), his weight was 19.6 kg (above 95th centile), height was 97.5 cm (between 50th and 75th centiles), and head circumference was 50.5 cm (between 50th and 75th centiles).

Development was globally delayed. He did not babble until the age of 2, and he only had two spoken words and one sign by the age of 3 years. Although he did sit independently at 6 months, walking was delayed until 20 months. He had some difficulty with the use of a spoon and fork, but he could use a cup and straw well. Atonic seizures developed at age 2½ years, but were well controlled with depakote. His EEG was markedly abnormal with a moderate degree of disturbance of cerebral function, maximally involving the posterior head regions. There were multifocal and independent areas of potential epileptogenicity noted maximally over the right posterior head region. Two clinical seizures were recorded during the EEG, suggestive of brief episodes of typical absence seizures.

Laboratory testing showed normal chromosomes, normal subtelomeric FISH, disomy for 15q11 FISH, and biparental disomy for chromosome 15. Chromosome 15 methylation analysis by Southern blot showed a mosaic methylation pattern. Although both maternal and paternal bands were observed, the methylated maternal band was significantly decreased compared to the unmethylated paternal band (Fig. 2). This finding was consistent between separate repeated samples. Sequencing of the 880 base pair imprinting center was normal.

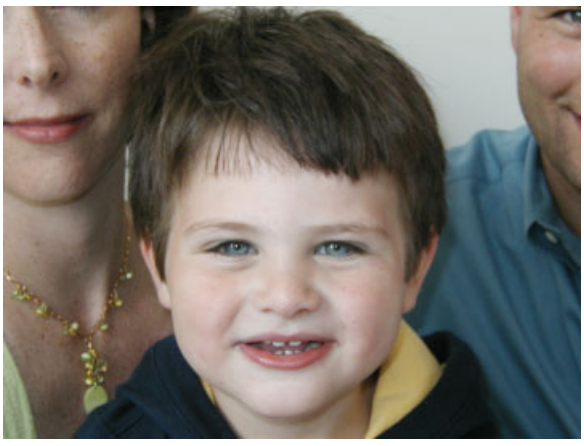

Fig. 1. Frontal facial view of Patient 1. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

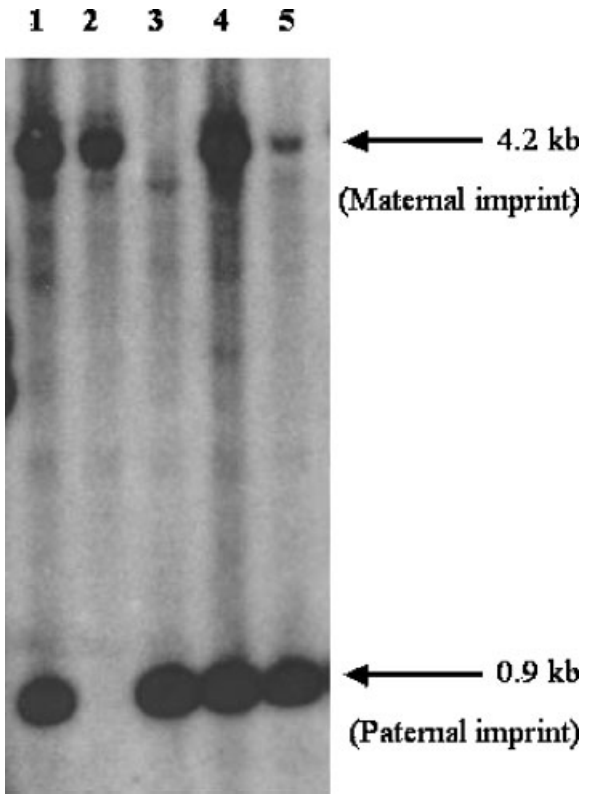

Fig. 2. Southern blot demonstrating mosaic methylation pattern. Lane 1 represents a sample from the father of Patient 1, lane 2 a control patient diagnosed with Prader–Willi syndrome, lane 3 a control patient with diagnosed Angelman Syndrome, lane 4 the mother of patient 1, and lane 5 Patient 1. The father and mother both have normal paternally and maternally imprinted methylation patterns. The patient has a mosaic pattern, with a predominantly paternal methylation pattern and a small amount of the maternal methylation pattern.

PATIENT 2

Patient 2 was delivered vaginally at term to his 32-year-old mother after an unremarkable pregnancy without exposures to known teratogens. Birth weight was 3.44 kg (between 10th and 25th centiles), length was 51.4 cm (approximately 75th centile), and head circumference was 33 cm (approximately 10th centile). Development has been globally delayed. He sat with support at 1 year, and was still unable to walk completely independently at age 4½ years. He had no words, but could point to items he wanted. Although he was unable to use utensils, he could finger feed. Strabismus that ultimately required surgery was noted at 6 months. He has not had clinical evidence of seizures, and two EEGs were normal. A third EEG at age 3 was abnormal, with intermittent paroxysmal spike wave discharges considered to be consistent with an evolving generalized epileptic syndrome. A brain MRI was normal.

At age 4½ years (Fig. 3), his height was 108 cm (75th centile), weight was 18.7 kg (75th centile), and head circumference was 48.2 cm (2nd centile), showing a slowing of head growth with post-natal microcephaly. His teeth appeared small and widely spaced. His demeanor was generally very happy. His gait had a wide stance.

Chromosome analysis, subtelomeric FISH panel, 15q11 FISH, DNA methylation studies and *UBE3A* mutation analysis were all normal. Genomic microarray-CGH utilizing the SignatureChip™ panel (Signature Genomics, Spokane, WA) was then performed as a screen for chromosome microdeletions. A single bacterial artificial chromosome (BAC) clone (RP11-466L14) showed a loss in copy number (data not shown). This clone encompasses a segment of at least 178 kb and includes a significant segment of the 5′ end of the *UBE3A* gene and upstream genomic sequence. Further analysis utilizing a chromosome 15-specific genomic microarray revealed loss for clone RP11-173H16 that partially overlaps and is immediately telomeric to clone RP11-466L14 (Fig. 4). Copy number losses detected by both clones were additionally confirmed by metaphase FISH analysis. The clones immediately proximal and distal to the above two clones were normal. An estimate of this loss including the 5′ half of the *UBE3A* gene and genomic sequence upstream of it is approximately 200 kb. The patient's mother was then tested with the SignatureChip™ in the same manner, revealing an identical loss of DNA copy number at the same BAC clone.

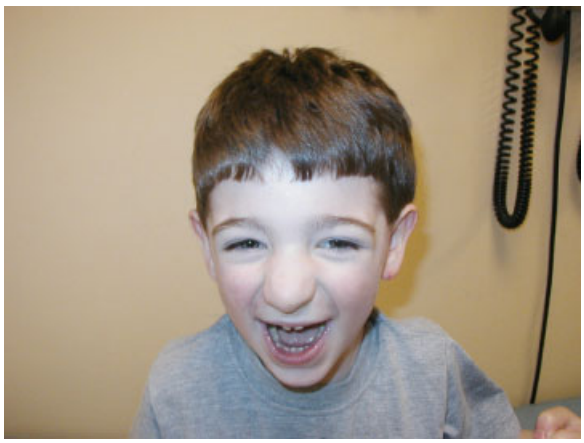

Fig. 3. Frontal facial view of Patient 2. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

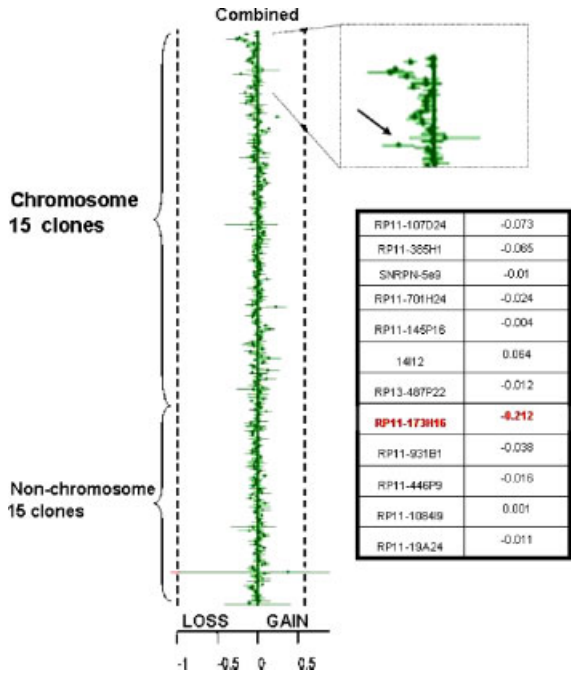

Fig. 4. Chromosome 15-specific array-CGH shows deletion of one BAC clone. The plot of the hybridization results Patient 2: Panel A: The combined $\log_2$ ratio for in a clone-by-clone order is shown. The combined ratio plot provides a final estimate of gain, loss, and no-change distribution for each clone. The dashed lines on the scale for the logarithmic plot (X-axis) indicate the position of −1.0 and +0.5, which are the theoretical values for single copy loss or gain, respectively. The plot is in clone-by-clone order starting (Y-axis: top to bottom) from 15 centromere to 15q telomere followed by chromosomes 1 to Y. The deletion in this case encompasses clone RP11-173H16 (inset; arrow; table:Log2 ratio significant for loss), and RP11-466L14 identified by SignatureChip (data not shown). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

DISCUSSION

Our two patients and recent reports by others reveal that AS can have a much broader phenotypic range than previously thought. Classically, the child with AS is thought to have very little language development, severe microcephaly, and delayed walking with ataxia. Generally, the diagnosis of AS may be dismissed as unlikely if a child has milder features. Specifically, if a child has more than a few words or does not have microcephaly, AS is generally thought to be relatively unlikely. As demonstrated in Patient 1, mosaic methylation can be associated with

a milder phenotype and can occur spontaneously. Patient 1 had a head circumference above the 50th centile even at the age of 3 and had learned two spoken words plus one sign by age 3 years.

Small *UBE3A* deletions, as seen in Patient 2, can be detected through new techniques such as microarray CGH. We believe cases of AS may be dismissed unless we continue to expand the available testing algorithm. Providing the correct diagnosis is invaluable for families who need to understand their child's condition, meet with other families who have similarly affected children, and understand recurrence risk. Recurrence risk is significantly higher (50%) for families in whom the mother carries a *UBE3A* mutation or deletion than for families who have de novo deletions or UPD (less than 1%). Thus, if one has high clinical suspicion for AS but traditional testing reveals normal results, one should still maintain suspicion for AS.

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