Letters to the Editor

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Prenatal Diagnosis and Carrier Detection for a Point Mutation in *UBE3A* Causing Angelman Syndrome

To the Editor:

Angelman syndrome (AS; MIM 105830) is a neurobehavioral disorder, characterized by severe mental retardation, absence of speech, seizures, and gait ataxia (Williams et al. 1995). AS is associated with maternal deficiency of the E6-AP ubiquitin-protein ligase (*UBE3A* gene; MIM 601623; Kishino et al. 1997; Matsuura et al. 1997). Molecular analysis can distinguish five classes of patients: those having a large deletion of a common interval of ~4 Mb on maternal chromosome 15q11-q13 (~70% of cases), those having paternal uniparental disomy (UPD) (3%–5%), those having imprinting mutations (7%–9%), those having intragenic mutations in *UBE3A* (2%–4%), and those having a clinical diagnosis of AS but with none of the above molecular abnormalities (10%–20%). The first three classes of AS patients demonstrate abnormal methylation patterns, by Southern blot analysis (Sutcliffe et al. 1994; Beuten et al. 1996) or by PCR amplification of bisulphite-treated DNA (Kubota et al. 1997), and are easily distinguished from the AS patients who are candidates to have intragenic mutations in *UBE3A*. Point mutations in *UBE3A* may be de novo or may be present on the paternal chromosome of mothers of affected children, which leads to a 50% recurrence risk in subsequent pregnancies, in the latter circumstance.

The *UBE3A* gene includes 10 exons that encode the major open reading frame and additional upstream exons that are primarily noncoding but may contribute to alternative isoforms (Yamamoto et al. 1997). Recent delineation of the exon/intron organization (Yamamoto et al. 1997) and the flanking intronic sequence of the major coding exons of the *UBE3A* gene (Matsuura et al. 1997), provide information for the design of PCR primers to amplify the coding exons and splice boundaries (see GenBank AF016703 to AF016708). We have used intronic PCR primers (available from the authors upon request; to be published elsewhere) to amplify and directly sequence genomic DNA, as described previously (Matsuura et al. 1997).

A family of mixed Ashkenazi and Iraqi Jewish descent with two children affected with AS was referred for genetic counseling. The mother was pregnant and requested prenatal diagnosis. Molecular analysis was performed by use of DNA isolated from the two affected children and their mother. Methylation analysis of the family by use of Southern blot hybridization was normal, and ruled out common deletion, UPD, and imprinting mutation as molecular abnormalities in this family (data not shown). Sequence analysis for the 10 major coding exons of *UBE3A* identified a nonsense mutation in exon 15. The mutation was a G→A substitution at nucleotide 2304 (numbering based on GenBank X98032), which caused a nonsense mutation at tryptophan codon 768 (W768X) at the protein level. Figure 1 illustrates the normal sequence from the father and the heterozygous mutation in the patient, with direct sequencing of amplified genomic DNA and the sequence for the cloned mutation. The W768X mutation was present in the mother of the affected children but was not present in her two siblings (II-1 and II-2 in fig. 1).

Cytogenetic analysis of cultured aminocytes revealed a normal female karyotype. Mutation analysis on DNA from cultured aminocytes indicated that the fetus did not inherit the W768X mutation from the mother. On the basis of this information, the mother continued the pregnancy and gave birth to a healthy female infant. The absence of the W768X mutation was confirmed by testing peripheral blood DNA from the infant, after birth.

In 70%–80% of cases of AS, the presence of the common ~4-Mb deletion, paternal UPD, or imprinting mutation, can be identified through the use of methylation analysis, FISH, and DNA marker studies (American Society of Human Genetics/American College of Medical Genetics Test and Technology Transfer Committee 1996). The recurrence risk is low for the common interstitial deletion and for UPD but can be extremely high in the case of chromosomal translocation or in some imprinting mutations (Buiting et al. 1998). The remaining 20%–30% of AS cases represent a difficult diagnostic problem, because mutation analysis for *UBE3A* is only available on a limited basis, and because the sequencing of all of the coding exons identifies a disease-

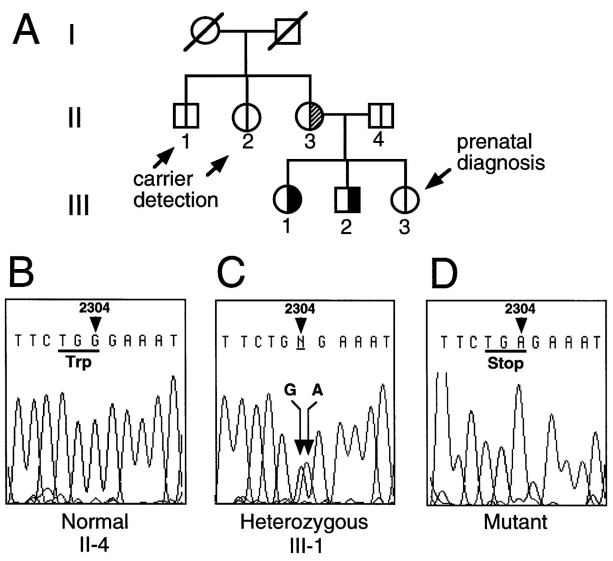

Figure 1 Prenatal diagnosis and carrier detection. *A,* Pedigree: half-blackened symbols (III-1 and III-2) indicate affected with AS and heterozygous for the W768X mutation, half-hatched symbol (II-3) indicates normal phenotype and heterozygous for the W768X mutation, and divided nonblackened symbols (II-1, II-2, II-4, and III-3) indicate normal phenotype and absence of the W768X mutation. *B* and *C,* Direct sequencing of genomic DNA for the normal father and for one of the affected children, respectively. *D,* DNA sequence for the cloned mutation.

causing mutation in only ~30% of the methylation-negative cases, in our experience. It is unknown whether the remaining AS patients have other molecular abnormalities or represent potential misdiagnoses. When disease-causing mutations are identified, as in the family reported here, the information is extremely valuable for genetic counseling and prenatal diagnosis.

In patients with typical AS clinical findings and normal methylation studies, the family history should be thoroughly investigated for the possibility of AS in distant maternal relatives, because the imprinted pattern of inheritance may result in the occurrence of multiple affected individuals, who are quite distantly related (Meijers-Heijboer et al. 1992). Although mutation studies for *UBE3A* would be valuable in all such families, they are particularly indicated in families with more than one individual affected with AS. In one report, mutations were found in 80% of multiplex families and in 14% of sporadic cases (Malzac et al. 1998), and we have found mutations in 75% and 23% for these two groups, respectively (P. Fang et al., unpublished data). In families where we have found disease-causing mutations, the index case has represented a de novo mutation in about half of the families, with the mother carrying the mutation in the remaining group (P. Fang et al., unpublished data). Thus, the risk of recurrence in families may be as high as 50% or may be relatively low, and the two circumstances are easily distinguished if a mutation is identified. In instances where no mutation is identified but where the clinical findings are typical of AS, considerable uncertainty prevails. Recurrence of AS is uncommon in this group but does occur.

To conclude, mutation analysis of *UBE3A* can be extremely informative for establishing a diagnosis of AS and for genetic counseling. If a disease-causing mutation is identified and is present in the mother, prenatal diagnosis is readily accomplished.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

GenBank, http://www.ncbi.nlm.nih.gov/Web/Genbank (for human genomic *UBE3A* sequences [AF016703–AF016708 and X98032])
Online Mendelian Inheritance in Man http://www.ncbi.nlm.nih.gov/Omim (for AS [MIM 105830] and for *UBE3A* [601623])

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