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A Novel *UBE3A* Truncating Mutation in Large Tunisian Angelman Syndrome Pedigree

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We identified in a large Tunisian pedigree a novel UBE3A frameshift mutation in exon 16 coding region, and we expect that the resulting UBE3A truncated protein in our patients is non-functional since the mutation implies the catalytic region of the enzyme. The family includes 14 affected patients born from four sisters. This mutation was found in all surviving affected individuals and their mothers pointing out the importance of genetic counseling possibility in Angelman syndrome (AS). All patients had severe mental retardation with epilepsy and microcephaly. Minor clinical expression variation was observed among the investigated patients. The severity of clinical expression is related to the detected molecular variation: deletion of 15 bp and insertion of 7 bp. These results are concordant with the gene expression observed in previously reported individuals with AS and truncated UBE3A protein. © 2009 Wiley-Liss, Inc.

Key words: Angelman syndrome; phenotype; UBE3A; mutation

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INTRODUCTION

Angelman syndrome (AS) is syndromic mental retardation with wide range of clinical features. Its incidence is estimated to be 1/10,000–1/20,000 [Clayton-Smith, 1993; Petersen et al., 1995] and it is characterized by a distinctive phenotype with four consistent signs that are: severe developmental delay; speech impairment; movement or balance disorder and behavioral uniqueness (especially frequent laugher and smiling) [Williams et al., 2006]. The behavioral characteristics of AS are striking and it is these which often prompt clinicians to consider the diagnosis [Clayton-Smith and Laan, 2003].

This phenotype is caused by a variety of genetic abnormalities [Jiang et al., 1999] involving the chromosomal region 15q11.2-q13 which contains a number of genes, including ubiquitin–protein ligase E3A (*UBE3A*), shown to cause AS [Kishino et al., 1997; Matsuura et al., 1997]. This region is subject to differential gene silencing via methylation during gametogenesis in the parents and this imprinting is controlled by a nearby imprinting center.

Familial cases can occur, due to mutations in the *UBE3A* gene or in the imprinting center. *UBE3A* mutations can be identified in around 75% of familial patients [Malzac et al., 1998; Fang et al., 1999; Clayton-Smith and Laan, 2003]. Several mutations have been reported [Kishino et al., 1997; Matsuura et al., 1997] and with the exception of a few of them [Kishino et al., 1997; Malzac et al., 1998; Tsai et al., 1998; Fang et al., 1999; Moncla et al., 1999] which have been found in more than one patient, most are unique.

We report on a large family of Tunisian origin with features consistent with AS, the disorder revealed to be caused by a new *UBE3A* mutation.

MATERIALS AND METHODS

Family and Clinical Description

We studied a large family originating from the northern west of Tunisia. Family investigation was pushed into four generations (Fig. 1) and showed many affected individuals with six affected males and eight affected females. They appear in only one generation (IV) and all of them except patient (IV-28) were born to cousin parents.

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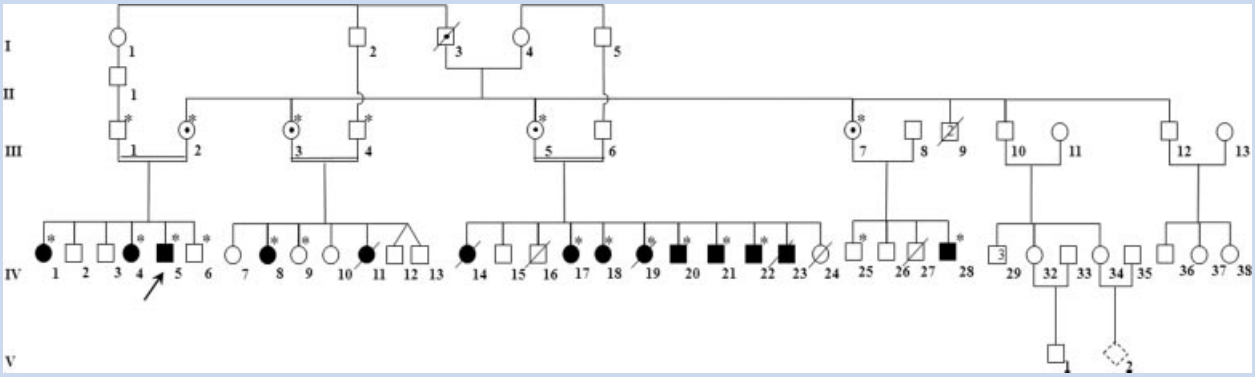

FIG. 1. Pedigree of the family. Affected males are represented by filled squares and affected females by filled circles. Dotted circles indicate female carriers. Individuals from whom DNA was available are tagged with asterisks.

Patient IV-5 (the propositus) had moderate mental retardation. He was born following a normal pregnancy, his birth weight was 3,000 g and his head circumference (OFC) was 28 cm, a very severe microcephaly. He had moderate psychomotor delay; he smiled at 6 weeks of age, sat unsupported at 7 months and walked independently when he was 18 months. He had disturbed sleep and developed seizures at days 10 and 15 of life than he was put under treatment. A cranial CT scan and an MRI were normal. He was examined at 7 years of age; his weight was 18 kg, 500, height 113.5 cm, and OFC 48 cm (−3.5 SD). He had subtle facial features including flat occiput, broad and protruding ears, prominent nose, and mild prognatism. He had also bilateral syndactyly of the 2nd and 3rd toes. His language skills were severely delayed and he could not articulate more than two syllables.

Patient IV-1, the older sister of the propositus was born at term following a normal pregnancy. She was severely delayed in all of her developmental milestones. She sat independently at 4 years and walked without assistance at 6 years of age. She had severe mental retardation. When examined at the age of 11 years, her head circumference was 49 cm (−2.8 SD) with flat occiput and prominent nose and mandible. She had also bilateral partial syndactyly of the 2nd and 3rd toes.

Patients IV-17, IV-18, IV-19, IV-20, IV-21, and IV-22 had severe psychomotor retardation (Table I), severe mental and speech impairment, and share mild microcephaly except for Patient IV-18 who has severe microcephaly with −5.3 SD. They walked with an unsteady gait, had a tendency to flap their hands, developed seizures before 3 years of age, and were hyperactive and always happy with frequent outbursts of laughter.

Molecular Genetic Analysis

Cytogenetic analysis. A karyotype to look for rare chromosomal rearrangements was performed.

Fluorescence in situ hybridization (FISH) was carried out using Vysis Angelman region probes that contains D15S1 at the 15cen region (spectrum green) and SNRPN at the 15q11-q13 AS critical region (spectrum orange).

Methylation analysis. 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. [1997]. Briefly, genomic DNA was treated 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 2% LE agarose gel (sigma) in 1× TBE buffer.

Sequencing analysis. Sequencing analysis included exons 9 and 16 of *UBE3A* gene shown to be hotspots of mutation [Fung et al., 1998]. Primers sequences are the following: forward 5′-ttgcaacagagtaaacatacatatt-3′ and reverse 5′-ctccatcattctccgaatctggt-3′ (exon 9 part one); forward 5′-ttgcaaagcgatgagcaagctaccc-3′ and reverse 5′-cactgaactgtatcatgatatc-3′ (exon 9 part two); forward 5′-actgatgtcctctctgtggttttgt-3′ and reverse 5′-tgcttgaggttgagcctttt-3′ (exon 16).

PCR was performed in a 50 µl reaction in thermocycler Gene Amp PCR System 9700 from Applied Biosystems, Foster City, CA. The amplicons were purified using a Promega Wizard SV Gel and PCR Clean-up System, and directly sequenced; sequencing reactions were prepared by means of ABI Big dye terminator v 3.1 cycle sequencing kit and separated by using the ABI 3130 Genetic Analyzer.

RESULTS

Cytogenetic analysis showed a normal karyotype for the propositus and FISH analysis detected no deletion in the affected siblings or their mothers. Molecular studies on samples from patients revealed a normal methylation pattern excluding the possibility of microdeletion encompassing the SNRPN locus, a mutation of the imprinting center or uniparental disomy.

Sequencing analysis of exon 16 of *UBE3A* gene showed differences between normal and patient sequences as it given in Figure 2. The propositus sequence had a deletion of 15 bp and insertion of 7 bp at the same position starting from the 86th nucleotide of exon 16 (3240_3255delinsAGATGTT). This mutation was found in all

TABLE I. Clinical Description of Investigated Patients

Patients	IV-5	IV-1	IV-21	IV-17	IV-22	IV-20	IV-18	IV-19
Age at last examination	7 years	18 years	15 years	24 years	13 years	16 years	23 years	19 years
Pregnancy	Hydramnios	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Childbirth	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Psychomotor development	Moderate delay	Severe delay	Severe delay	Severe delay	Severe delay	Severe delay	Severe delay	Severe delay
Sitting without support	7 months	4 years	1 year	2 years	1 year	NA	NA	NA
Walk	18 months	6 years	4 years	3 years	3 years	4 years	18 months	18 months
Speech	Few syllables	Few syllables	Few syllables	Few syllables	Few syllables	Few syllables	Few syllables	Few syllables
Dysmorphic features								
Convex nasal ridge	+	+	+	+	Moderately			+
Round nasal tip				+	+			+
Flat forehead	+		+	+				
Mild ptosis	+							
Narrow palpebral fissures			+	+	+	+	+	+
Flattened face					+			
Mandible prognatism	+	+						
Wide mouth			+	+	+		+	+
Large incisors		+				+	+	+
High narrow palate			+					
Large ears	+				+	+		
Protruding ears	+							
Short philtrum	+	+	+	+	+	+	+	+
Bilateral syndactily of 2nd and 3rd toes	+	+		+			+	+
Obesity					+			
Microcephaly	−3.5 SD	−2.7 SD	−2.5 SD	−3 SD	−2.2 SD	−1.6 SD	−5.3 SD	−2.7 SD
Seizures	+	+	+	+	+	+	+	+
Ataxia		+	+	+	+	+	+	+
Outbursts of laughter		NA	+	+	+	+	+	+
Imaging investigations	Normal MRI	NI	NI	NI	NI	NI	NI	NI

NA, non-available; NI, non-investigated; SD, standard deviation.

affected individuals and their mothers but not in normal siblings and fathers.

DISCUSSION

We characterized a novel *UBE3A* deletion–insertion mutation in a large Tunisian family comprising several individuals with AS. According to the Human Genome Mutation Database (www.hgmd.cf.ac.uk), this is the first report of small insertion–deletion mutation in the *UBE3A* gene. The propositus had moderate intellectual disabilities when examined at the age of 7 years and he had significant microcephaly. Clinical investigation in the remaining affected individuals, especially siblings IV-17, IV-18, IV-19, IV-20, IV-21, and IV-22 (Fig. 1), who presented outbursts of inappropriate laugher, suggests the diagnosis of AS. In addition, although one might attribute the recurrence of the phenotype in this family to autosomal recessive inheritance, it is known that imprinting inheritance can lead to an affected/non-affected ratio similar to or higher than the 25% expected for an autosomal recessive condition.

We note some variation in phenotypic aspects between affected members of this family and especially in individuals IV-5 (severe congenital microcephaly) and IV-18 (severe microcephaly) which may suggest segregation of another genetic disorder in the kindred since congenital microcephaly is not observed in AS.

UBE3A codes for E6AP enzyme, an E3 ubiquitin–protein ligase which has an important role in the processes of ubiquitination [Ciechanover et al., 2000]. Its biological activity is believed to be defined largely by its carboxyl terminal end; a highly conserved domain (called HECT for homologous to E6-AP carboxyl terminus) that performs ligase activity [Verdecia et al., 2003] and which is located in the 3′ end of exon 9 and exons 10–16 [Fung et al., 1998].

Most of the AS-associated mutations lie within this domain with hotspots observed in exons 9 and 16 and striking preponderance of frameshift and nonsense mutations (Table II).

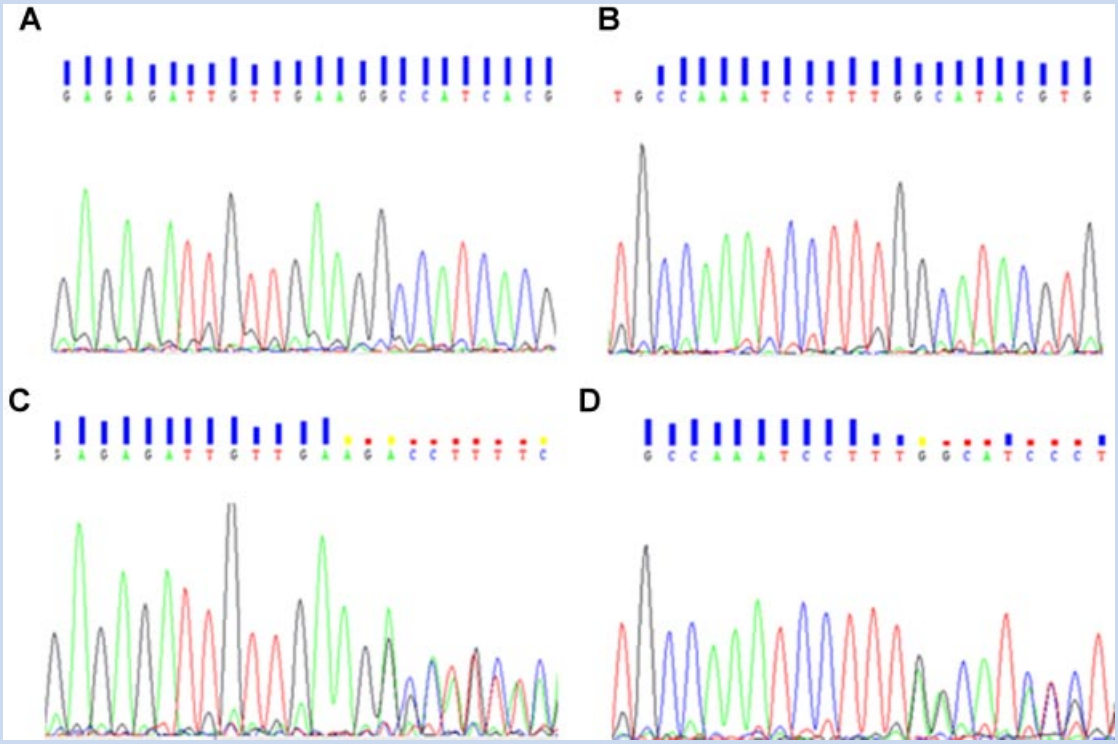

FIG. 2. Sequence chromatograms of a control individual (upper traces) and affected family member IV-5 (lower traces) showing a partial sequence of exon 16. Left traces (A and C) correspond to sequencing with forward primer of exon 16 while right traces (B and D) correspond to sequencing with reverse primer. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The deletion–insertion mutation identified in our family (3240_3255delinsAGATGTT) results in a frameshift that generates a stop codon at position 843 of the protein. We predict that the resulting UBE3A truncated protein in our patients would be non-functional since the mutation would disrupt the catalytic region of the enzyme. We think that the mutant protein will be defective for catalyzing ubiquitin transfer since it lacks the last six amino acids defined by Huibregtse et al. [1995] and required for this function. These data are consistent with the inheritance of a causative *UBE3A* mutation and confirm the diagnosis of AS in these siblings.

Exploration for this mutation in the remaining maternal relatives of the propositus, particularly individuals III-10 and III-12 (Fig. 1) and their offspring, would be very useful to determine carrier status. If mutations are identified, a prenatal diagnosis could be offered for carrier mothers.

Our patients display a clinical picture similar to that reported in AS patients with *UBE3A* truncating mutations. *UBE3A* mutations resulting in frameshifts and/or premature truncations give a more severe phenotype than missense mutations or short in-frame deletions that still allow the translation of a full-length protein [Fang et al., 1999]. Matsuura et al. [1997] and Rapakko et al. [2004] described *UBE3A* missense mutations with features milder than those of classical AS patients.

Like other previously reported patients with *UBE3A* mutation, the severity of the phenotype in our family tends to fall in the middle between clinical expression due to large 15q11.2-q13 deletions and that related to uniparental disomy. Patients with *UBE3A* mutations like those with the microdeletion share the common clinical features of seizures, absent speech and microcephaly whereas they have better motor and communication skills, as is also observed for those with uniparental disomy [Lossie et al., 2001; Clayton-Smith and Laan, 2003; Williams, 2005].

We note also the unusual large size of this family counting 14 affected individuals; it is evident that the propositus' mother and all her sisters (III-2, III-3, III-5, and III-7; Fig. 1) inherited the mutation from their father (I-3).

However, despite an exhaustive family history investigation it is difficult to know if the mutation appeared at first sight with this individual or in an earlier generation. Because of the imprinting inheritance, *UBE3A* mutations could be inherited through several generations without any phenotypical manifestation when transmitted from fathers to sons. We should note also that the paternal origination of this type of mutation is not recurrent in AS since paternal inherited *UBE3A* mutations are more likely to be single nucleotide substitutions while multiple nucleotide deletions and insertions occur generally de novo [Camprubi et al., 2009].

Detected mutations in large AS families' points out the importance of the genetic counseling availability in AS and highlights the necessity of meticulous family history investigations.

TABLE II. Reported UBE3A Gene Mutations

Mutation	Site	Form of AS	Refs.
Frameshift			
645insA	Exon 8	Sporadic (mother mosaic)	Malzac et al. [1998]
762-763insGA	Exon 8	Familial	Baumer et al. [1999]
856delG	Exon 8	Familial	Fang et al. [1999]
897insA	Exon 9	Familial	Russo et al. [2000]
904del5	Exon 9	Sporadic (de novo)	Fang et al. [1999]
946delAG	Exon 9	Sporadic (de novo)	Laan et al. [1999]
980delAG	Exon 9	Familial	Fang et al. [1999]
1161dup4	Exon 9	Familial	Hitchins et al. [2004]
1296insT	Exon 9	Sporadic (de novo)	Baumer et al. [1999]
1461del14	Exon 9	Familial	Malzac et al. [1998]
1522delG	Exon 9	Familial	Malzac et al. [1998]
1552delA	Exon 9	Sporadic (de novo)	Fang et al. [1999]
1559del7	Exon 9	Sporadic (de novo)	Fang et al. [1999]
1694del4	Exon 9	Familial (large pedigree)	Fang et al. [1999]
1930delAG	Exon 9	Sporadic (de novo); sporadic (maternal)	Matsuura et al. [1997], Fung et al. [1998], Lossie et al. [2001], Rapakko et al. [2004]
1965delT	Exon 9	Sporadic (de novo)	Hitchins et al. [2004]
1993del5	Exon 9	Familial	Hitchins et al. [2004]
2037del10	Exon 9	Familial	Malzac et al. [1998]
Duplication of GAGG	Exon 10	Familial	Molfetta et al. [2004]
2230del26insA	Exon 10	Sporadic (de novo)	Malzac et al. [1998]
2376delG	Exon 11	Sporadic (de novo)	Baumer et al. [1999]
2527insA	Exon 12	Familial	Malzac et al. [1998]
2544insA	Exon 12	Sporadic (present in mother)	Russo et al. [2000]
2567ins4	Exon 12	Sporadic (de novo)	Fang et al. [1999]
3027insT	Exon 16	Familial	Ouweland van der et al. [1999]
3033insA	Exon 16	Familial	Malzac et al. [1998]
3038ins8	Exon 16	Sporadic (de novo)	Russo et al. [2000]
3076ins4	Exon 16	Sporadic (parents not tested)	Ouweland van der et al. [1999]
3086ins4	Exon 16	Sporadic (de novo)	Russo et al. [2000]
3086ins5	Exon 16	Sporadic (de novo)	Kishino et al. [1997], Malzac et al. [1998]
3092delA	Exon 16	Sporadic (de novo)	Lossie et al. [2001]
3093delAAGA	Exon 16	Sporadic (de novo)	Fang et al. [1999], Lossie et al. [2001], Rapakko et al. [2004], Hitchins et al. [2004]
3094dup5	Exon 16	Sporadic (de novo)	Hitchins et al. [2004]
3120ins16	Exon 16	Sporadic (de novo)	Baumer et al. [1999]
3240_3255delinsAGATGTT	Exon 16	Familial (large pedigree)	Present study
Nonsense			
1500G → A (W305X)	Exon 9	Sporadic (present in mother and maternal grandfather)	Fang et al. [1999]
1835C → T (R417X)	Exon 9	Sporadic (de novo)	Matsuura et al. [1997]
1085GT (E167X)	Exon 9	Sporadic (de novo)	Russo et al. [2000]
2030C → T (R482X)	Exon 9	Sporadic (de novo); probably familial	Malzac et al. [1998], Russo et al. [2000]
2033A → T (R483X)	Exon 9	Familial	Lossie et al. [2001]
2185T → G (Y533X)	Exon 9	Familial	Fang et al. [1999]
2890G → A (W768X)	Exon 15	Familial	Tsai et al. [1998]
Missense			
648G → A (C21Y)	Exon 8	Sporadic (present in mother)	Matsuura et al. [1997]
902A → C (T106P)	Exon 9	Sporadic (mother mosaic)	Rapakko et al. [2004]
975T → C (I130T)	Exon 9	Sporadic (de novo)	Rapakko et al. [2004]
1631T → C (S349P)	Exon 9	Familial	Malzac et al. [1998]
2102C → T (R506C)	Exon 9	Sporadic (de novo)	Baumer et al. [1999]
2997T → A (I804K)	Exon 15	Sporadic (de novo)	Fang et al. [1999]
Splicing mutations			
IVS14-2A>G		Familial (mother mosaic)	Hosoki et al. [2005]
c.1693+1G>A	Intron 10		Sartori et al. [2008]
IVS9-8A → G	intron 9	Familial	Kishino et al. [1997]
Amino acid deletion/insertion			
2855del12 (YTRD757D)	Exon 14	Familial	Hitchins et al. [2004]
2992ins3	Exon 15	Familial	Malzac et al. [1998]
2929del3 (F782Δ)	Exon 15	Sporadic (de novo); familial	Russo et al. [2000], Fang et al. [1999], Hitchins et al. [2004]
3142del15	Exon 16	Sporadic (de novo)	Fang et al. [1999]

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