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Mutation Analysis of UBE3A in Angelman Syndrome Patients

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Summary

Angelman syndrome (AS) is caused by chromosome 15q11-q13 deletions of maternal origin, by paternal uniparental disomy (UPD) 15, by imprinting defects, and by mutations in the UBE3A gene. UBE3A encodes a ubiquitin-protein ligase and shows brain-specific imprinting. Here we describe UBE3A coding-region mutations detected by SSCP analysis in 13 AS individuals or families. Two identical de novo 5-bp duplications in exon 16 were found. Among the other 11 unique mutations, 8 were small deletions or insertions predicted to cause frameshifts, 1 was a mutation to a stop codon, 1 was a missense mutation, and 1 was predicted to cause insertion of an isoleucine in the hect domain of the UBE3A protein, which functions in E2 binding and ubiquitin transfer. Eight of the cases were familial, and five were sporadic. In two familial cases and one sporadic case, mosaicism for UBE3A mutations was detected: in the mother of three AS sons, in the maternal grandfather of two AS first cousins, and in the mother of an AS daughter. The frequencies with which we detected mutations were 5 (14%) of 35 in sporadic cases and 8 (80%) of 10 in familial cases.

Introduction

Angelman syndrome (AS [MIM 105830]), which is characterized by severe mental retardation, lack of speech, seizures, easily provoked smiling and laughter, ataxia, and sleep disorder, is caused by a number of different genetic alterations involving chromosome 15q11-q13 (Williams et al. 1995). The phenotypic expression of these chromosome 15 abnormalities is completely dependent on the parent from whom the aberration is inherited. For instance, maternally inherited deletions of 15q11-q13 are responsible for 70% of AS cases, whereas paternally inherited deletions of the same region cause the phenotypically distinct Prader-Willi syndrome (PWS). Some of the remaining 30% of AS cases are caused by paternal UPD 15 (2%–3% of total AS cases) and by imprinting defects with abnormal methylation and gene expression patterns (2%–5%). Among the remaining nondeletion/non-UPD/non–imprinting-defect AS patients, there are many instances of familial recurrence of AS that show an imprinted pattern of inheritance, with phenotypic expression only after maternal transmission. We and others have detected point mutations in the UBE3A gene in some of these patients (Kishino et al. 1997; Matsuura et al. 1997), which have led to the conclusion that this is the 15q11-q13 gene responsible for the AS phenotype.

Initial analysis of gene expression in lymphoblastoid cell lines and fibroblasts from AS and PWS patients indicated that UBE3A is not imprinted (Nakao et al. 1994). However, several subsequent reports have provided evidence for brain-specific imprinting of UBE3A in both humans and mice (Albrecht et al. 1997; Rougeulle et al. 1997; Vu and Hoffman 1997). Using mice with paternal UPD for the region of chromosome 7 containing UBE3A, Albrecht et al. (1997) showed that its imprinting is region specific, with almost undetectable levels of expression in regions of the hippocampus, in cerebellar Purkinje cells, and in mitral cells of the olfactory bulb in the UPD mice (Albrecht et al. 1997). Maternal inheritance of UBE3A mutations therefore results in phenotypic abnormalities limited to the brain because of brain-specific preferential expression of the maternal allele.

How deficiency of the UBE3A protein in the brain leads to the AS phenotype is unknown. UBE3A, as a ubiquitin-protein ligase, catalyzes transfer of ubiquitin

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Table 1

Primer Pairs Used for SSCP Analysis

Exon and Primer	Sequence	Region	Annealing Temperature (°C)	Product Size (bp)
4:				
7-1A	TGTATTTCACTTTACAATGAC	Intron	52	213
7-1B	TAAAGTGTTCTAACCAAAGG	Intron		
5:				
723-A	TTTCTCTAGAAGTTTTTATAAC	Intron	52	197
723-B	ATCGCAGAAAATATGATCAC	Intron		
7:				
E61-A	GCTAACTGTTTCTCAATTGC	Intron	52	105
E61-B	ATAAGAACCACAGTCTCAAC	Intron		
8:				
E6-8A	CACTGTGCTTATTGTTTGAATG	Intron	55	154
E6-8B	TATCCATACGAAGAAAAGTTG	Exon		
E6-8C	ATGAGTTTTGTGCTTCCTGT	Exon	55	152
E6-8D	TTTCGAGTTCTCAAGGTAAG	Exon		
E6-8E	CCTCCAAGAAAGGAGCAAGC	Exon	55	155
E6-8F	TGGTTTTCAGGCAACAATTCTC	Intron		
9:				
E6-9K	AGAACTTTTTGCAACAGAGTA[a]	Intron	55	164
E6-9L	CCAATAACACGGATTAAAGG	Exon		
E6-9M	GAAATTCTTGAATTATGTAGAGA	Exon	55	197
E63-B	GCAGCAGAACATGCAGCTTT	Exon		
E63-C	GATGAAGACAAAGATGAAGAT	Exon	52	153
E63-D	ATCCACAGACACATCATCAG	Exon		
E63-E	GACAACAATTTGCAAAAATTAG	Exon	52	151
E63-F	AGTCACATTCCACGTTAGGT	Exon		
E63-G	GCCTTTCTCAATGCACTTGT	Exon	52	153
E63-H	AGCCATTTCCAGATATTCAG	Exon		
E63-I	AGAATAGAAATCTCCACAGTC	Exon	52	150
E63-J	TCTCCATCATTCTCCGAATC	Exon		
E63-K	GTGGTCTAAATACAATGCAG	Exon	55	158
E63-L	AGTAAACCATTTTCAAGCAC	Exon		
E63-M	GATGCCATTGTTGCTGCTTC	Exon	55	152
E63-N	TCTTCTCCCAAAAGTTCCTG	Exon		
E6-9A	CCCATCCCTGAGTCCAGCGA	Exon	55	165
E6-9B	CAGTGGTTCATTAATAAACTCT	Exon		
E6-9C	ATTGTCGAAAACCACTTATC	Exon	55	163
E6-9D	CCCAAATTCTTTGTGACAGCA	Exon		
E6-9E	ATGACATGTCCCTTTATATTG	Exon	55	158
E6-9F	TCACGTCTAACTTTGAGTCT	Exon		
E6-9G	TTAGTTCAAGGACAGCAGTT	Exon	55	119
E6-9H	CCCCATTATTAGGTTTTTAATCT	Intron		
10:				
E6-10A	GCAATCATCTTCTTTTCATGTT	Intron	55	217
E6-10B	CGACACCATAATCACATTAC	Intron		

(*Continued*)

Table 1

(Continued)

Exon and Primer	Sequence	Region	Annealing Temperature (°C)	Product Size (bp)
11:				
E6-11A	AGTCCTTAATAAAATACAAAAGT	Intron	55	150
E6-11B	ACCCAGTACTATGCCAATCA	Exon		
E6-11C	AACTGAGGGTCAGTTTACTC	Exon	55	175
E6-11D	GAATTAAAAAAATGACAAAGAAC	Intron		
12:				
E6-12A	TGTTGTATTTTGTAGTTCTATGG	Intron	52	172
E6-12B	AGATCATACATCATTGGGTTAC	Exon		
E6-12C	GATCACTTTCCAGATATCACAG	Exon	52	143
E6-12D	TTAATGAAGAGACAAAATGTGAC	Intron		
13:				
E6W-A	GAAGTTCTTGTGATTAATGT[b]	Intron	52	137
E6W-B	CTTTAAGGGAGATTCATTGG	Exon		
E6W-C	AAAACAGTTCAAGGCTTTTC	Exon	52	145
E6W-D	CGATACATGACTTTTTGCAG	Intron		
14:				
E6-14F	AGTAGTATAGCAGATAACTAAGAC	Intron	52	164
E6-14R	CCCTTTGGTGAATCAAATCTTCC	Intron		
15:				
E6Y-A	TGAATGCCAAACTGAAACCA	Intron	52	130
E6Y-C	CCCACAGGTGCTCTGTCT	Exon		
E6Y-B	TTCGGTAGGTATACAGTCAC	Intron	52	147
E6Y-D	AACAGAAAAGACTCTTCTTG	Exon		
16:				
E6Z-A	CCATGACTTACAGTTTTCCT	Intron	55	277
E6Z-B	TGGGACACTATCACCACCAA	3′ UTR		

[a] The primer used in this study had an extra G erroneously inserted after the second base of this primer; the sequence shown in this table reflects the correct genomic sequence (H. Kokkonen 1997, personal communication).

[b] The primer used in this study had an extra T erroneously inserted after the fourth base of this primer; the sequence shown in this table reflects the correct genomic sequence (H. Kokkonen 1997, personal communication).

to substrates, which are thereby targeted for degradation by the proteasome (Huibregtse et al. 1993*a*). The two known substrates of UBE3A are p53, whose ubiquitination by UBE3A is dependent on the E6 protein of high-risk human papillomaviruses (HPVs), and HHRAD23A, a homolog of the yeast Rad23 protein, whose ubiquitination is E6 independent (Kumar et al. 1997). There is no evidence for ubiquitination of p53 by UBE3A in cells not infected with HPV, and it is unknown whether HHRAD23A plays a role in the pathogenesis of AS.

In this report, we describe mutation analysis of the UBE3A coding region in a group of 35 sporadic and 10 familial AS cases. We have found mutations in 13 of these cases. Our results demonstrate that alterations throughout the UBE3A coding region can cause AS and that these mutations can include chain-terminating, missense, and amino acid–insertion mutations. We also provide evidence of mosaicism for UBE3A mutations in the mothers and in the grandfathers of AS individuals.

Material and Methods

Patients

Patients either were examined by one of us or were referred by clinical geneticists, with a diagnosis of either definite or probable AS. Deletion, UPD, and imprinting mutations had been excluded in all of the patients by some combination of FISH analysis, methylation analysis, and polymorphism analysis for UPD. Informed consent for mutation analysis was obtained from all families.

SSCP Analysis

We performed SSCP analysis using genomic DNA from patients and the primers listed in table 1. PCR reactions for SSCP analysis were carried out with the annealing temperatures listed in table 1. PCR products from exon 16 were cleaved with *Msp*I before electrophoresis. Products were separated on 0.5 × Mutation Detection Enhancement gels (A-T Biochemical) containing 10% glycerol at room temperature, or without glycerol at 4°C, and were visualized by autoradiography. All SSCP band shifts were verified by repetition of the PCR and SSCP. Abnormal bands were cut out, eluted in water, and reamplified by PCR with the original primers for sequencing. Parents and other family members were tested for known UBE3A mutations by SSCP analysis.

Linkage Analysis

Linkage analysis in family 5 was done as described elsewhere (Wagstaff et al. 1993).

DNA Sequencing

PCR products were sequenced on an ABI 373A or ABI 377 automated DNA sequencer. In cases where both PCR primers were from the same exon, the sequence of the mutant allele was compared with the UBE3A sequence and with the sequences of two processed pseudogenes of UBE3A (Kishino and Wagstaff 1998), to verify that the mutation affects UBE3A and not one of the pseudogenes.

Results

We analyzed 45 cases with diagnoses of either definite or probable AS, for mutations in the coding regions of UBE3A. Thirty-five of the nondeletion/non-UPD/non–imprinting-defect AS cases were sporadic, and 10 were from families with more than one AS individual (fig. 1). The SSCP analysis included exons 4, 5, and 7–16, which include the known open reading frames of UBE3A (Yamamoto et al. 1997; Kishino and Wagstaff 1998). Among this group, mutations were detected in 13 cases: there were 12 unique mutations, with 1 mutation present in two individuals (table 2). (These mutations include the two described by Kishino et al. 1997.) Eight of the mutations were insertions, deletions, or splice mutations predicted to cause frameshifting and premature termination of translation. The splice mutation in intron 9 (family 2) was shown to cause abnormal splicing of UBE3A RNA in a lymphoblastoid cell line established from one of the brothers (data not shown). One mutation (family 4) produced a stop codon. The mutation in family 5 is predicted to cause a serine to proline change at position 349, and another mutation (family 7) is predicted to cause insertion of an isoleucine at position 802. This insertion mutation was not present in 82 unrelated normal control individuals. Mutations were detected in exons 8, 9, 10, 12, 15, and 16.

In families with more than one AS individual (i.e., families 2, 3, 6, 7, 8, 10, and 11), all affected individuals in a family were heterozygous for the same mutation, as were their phenotypically normal mothers (for family 5, see below). The mothers' normal phenotypes are presumably due to inheritance of the UBE3A mutations from their fathers. This transmission of a UBE3A mutation from father to normal daughter to AS child was shown directly in family 11 (data not shown).

Two variants were identified that are probably not pathogenic. Three patients were heterozygous for the A178T missense mutation; in the patient described by Matsuura et al. (1997) and in one patient examined by us, the mutation was inherited from the father, indicating that it is not causative of AS. The two AS sisters in family 8, in addition to having the 2527insA mutation, were both heterozygous for a G→A transition at position 702, leading to R39H substitution. This base change was inherited from their father.

Family 5, in which the S349P missense mutation was detected in three AS brothers, had originally been analyzed with polymorphic microsatellite markers flanking UBE3A. This analysis (fig. 2) gave the unexpected result that two AS brothers shared the same maternal haplotype with an unaffected brother; the third AS brother had a crossover between D15S11 and GABRB3, which flank UBE3A. SSCP analysis with primers E63-K and E63-L showed the same abnormal band in the three AS brothers but not in their normal siblings. The phenotypically normal mother showed a faint abnormal band (<5% of wild-type band density), demonstrating mosaicism for the mutation. Sequence analysis of the abnormal band showed a T→C transition at position 1631, predicted to change serine to proline at position 349. The mother's apparent somatic and germline mosaicism accounts for the presence of the same maternal haplotype in normal and AS siblings.

In family 11, in which two brothers and their maternal first cousin with AS were heterozygous for a 14-bp deletion in exon 9, their normal mothers also were heterozygotes for the same mutation. Their maternal grandfather's blood DNA showed only a very faint SSCP band corresponding to the mutation (data not shown), indicating that he also is mosaic, both in blood and germline, for this deletion. Similarly, in family 12, with a single affected child bearing a single-base insertion in exon 8, the mother showed a very faint SSCP band corresponding to the mutant allele, indicating mosaicism. In the other four families with a single affected child, the mothers were not heterozygotes, and there was no evidence of mosaicism.

We have compared clinical characteristics of AS in-

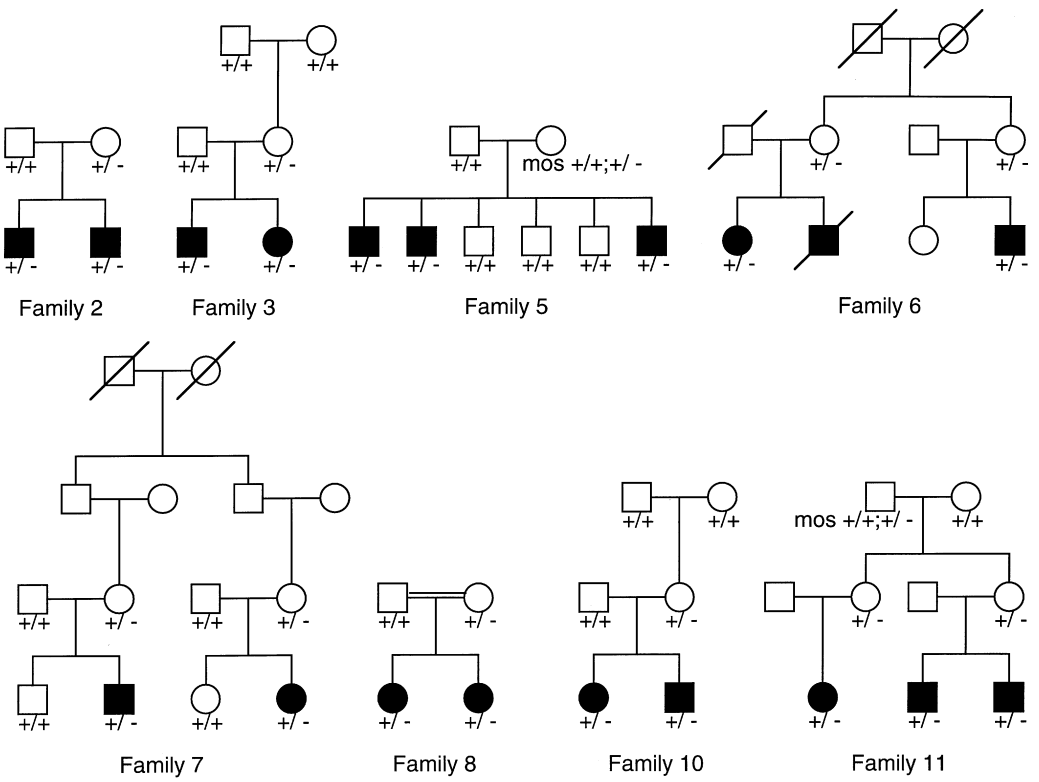

Figure 1 Pedigrees of familial AS cases. Genotypes are shown for affected individuals and for unaffected individuals who have been tested. Mutations are denoted by the minus sign (−); the mutation in each family is described in table 1. Mosaicism is indicated by "mos."

dividuals from 11 families with UBE3A mutations predicted to cause premature chain termination (families 1–4, 6, and 8–13) with those of AS individuals with either missense or amino acid–insertion mutations (families 5 and 7). Approximately half of the individuals in each group showed microcephaly (occipitofrontal circumference less than second percentile). In both groups, almost all AS individuals had a clinical history of seizures. The frequency of AS individuals who walked independently before 24 mo of age was 5 (28%) of 18 in the chain-terminating–mutation group and 0 of 5 in the missense/amino acid–insertion group.

Discussion

We have found intragenic mutations of UBE3A in 13 individuals and families with AS not caused by large deletion, UPD, or imprinting mutations. These mutations, taken together with the balanced inversion breakpoint between UBE3A exons 1 and 2 (Greger et al. 1997; Kishino et al. 1997), indicate that intragenic alterations spanning essentially the entire 120-kb UBE3A gene cause AS when transmitted maternally. Although there is a complex pattern of alternative splicing in the 5′ UTR of UBE3A (Rougeulle et al. 1997; Yamamoto et al. 1997; Kishino and Wagstaff 1998), all of these mutations are predicted to affect all of the known splice products.

Most of the mutations that we have found are predicted to lead to premature stop codons in UBE3A mRNA. These mutations probably lead to their phenotypic effects both by reducing UBE3A mRNA concentrations and by causing any translation products to be catalytically inactive because of the importance of the extreme C terminus of the UBE3A protein for its catalytic function (Huibregtse et al. 1995). The two non–chain-terminating mutations include S349P in three AS brothers and I802ins in two cousins. The S349P change is within the minimal region of UBE3A that can direct E6-dependent association with p53 (Huibregtse et al. 1993*b*). Although there is no evidence for p53 as a UBE3A substrate in non–HPV-infected cells, this region is likely to play a role in the binding of non–E6-dependent substrates whose ubiquitination may play a role in the pathogenesis of AS. The isoleucine duplication (I802ins) lies within the hect domain that is conserved among a number of E3 ubiquitin-protein ligases from diverse organisms (Huibregtse et al. 1995). The hect domain functions in E2 binding and in ubiquitin transfer (Kumar et al. 1997). This duplication is 16 amino acids N-terminal to the cysteine residue to which ubiquitin is

Table 2

UBE3A Mutations in AS Individuals and Families

Family	Form of AS	Site	Sequence Change	Predicted Consequence	Affected Individual(s)
1	Sporadic (de novo)	Exon 16	3086ins5	Frameshift	Proband
2	Familial	Intron 9	IVS9-8A→G	Frameshift	Two brothers
3	Familial	Exon 9	1522delG	Frameshift	Brother and sister
4	Sporadic (de novo)	Exon 9	2030C→T	R482X	Proband
5	Familial	Exon 9	1631T→C	S349P	Three brothers
6	Familial	Exon 16	3033insA	Frameshift	Two maternal first cousins
7	Familial	Exon 15	2992ins3	I802ins	Two maternal second cousins
8	Familial	Exon 12	2527insA	Frameshift	Two sisters
9	Sporadic (de novo)	Exon 10	2230del26insA	Frameshift	Proband
10	Familial	Exon 9	2037del10	Frameshift	Brother and sister
11	Familial	Exon 9	1461del14	Frameshift	Two brothers and maternal first cousin
12	Sporadic (maternal mosaic)	Exon 8	645insA	Frameshift	Proband
13	Sporadic (de novo)	Exon 16	3086ins5	Frameshift	Proband

NOTE.—Nucleotide and amino acid positions are according to GenBank U84404.

covalently linked before its transfer to substrates. However, although one of the two isoleucine residues at this site in the normal protein is conserved among many hect family members, this portion of the protein is otherwise not highly conserved in the hect family. Determining the effects of these amino acid–sequence changes on UBE3A function will be possible with use of in vitro assays with E6-dependent and independent substrates. As more UBE3A substrates are identified, these mutations may facilitate the determination of which substrates are important in the pathogenesis of AS.

We have found mutations in 5 (14%) of 35 sporadic AS cases and in 8 (80%) of 10 familial cases. These cases either were examined by one of the authors or were considered by referring physicians to have either a definite or a probable clinical diagnosis of AS. The reason for this significant discrepancy between mutation frequencies in the sporadic versus familial cases is unclear. It is unlikely to be because of different efficiencies of mutation detection in the two groups: we have found deletions, insertions, and base substitutions in both groups, and there is no reason to suspect that SSCP is more sensitive to the mutations in familial cases than to those in sporadic cases. A second possibility is that the diagnosis of AS is more likely to be accurate in the familial cases than in the sporadic cases. This could be true if one or more disorders unrelated to 15q11-q13 can cause an AS-like phenotype and can occur predominantly in sporadic form. There is no published evidence for locus heterogeneity in AS.

We have found evidence for mosaicism in three families: in one case, three of the six sons of a mosaic mother were affected with AS; in the second case, two of the three daughters of a mosaic male inherited the mutation and had AS offspring; and in the third case, one AS child was born to a mosaic mother. It is important to consider

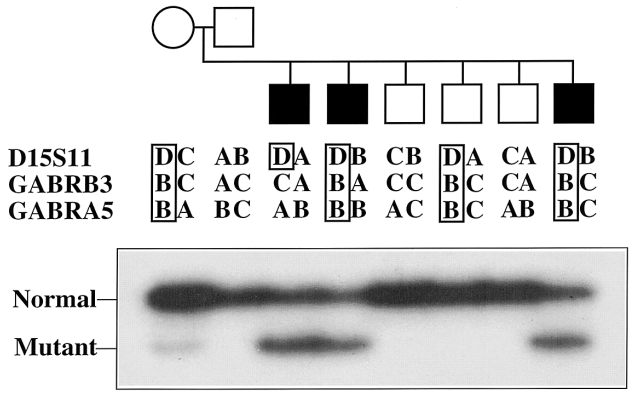

Figure 2 Linkage and SSCP results in family 5. The pedigree, with three AS brothers and three normal brothers, is shown together with genotypes for loci D15S11, GABRB3, and GABRA5. The maternal haplotype DBB present in two AS brothers and one normal brother is boxed; the third AS brother has a crossover between D15S11 and GABRB3 and has only the centromeric marker D of that haplotype. The lower panel shows a portion of the SSCP gel on the family, created by the use of primers E63-K and E63-L and a gel run at 4°C with no glycerol. The three normal brothers and the father have only the upper (normal) band; the three AS brothers have upper and lower (mutant) bands of equal intensity; and the mother has a lower band of <5% of the intensity of the upper band, demonstrating mosaicism for the UBE3A S349P mutation.

the possibility of mosaicism when providing genetic counseling in families with nondeletion AS offspring. In particular, the fact that an AS child and a normal sibling have inherited the same maternal haplotype does not exclude the possibility of recurrence of AS to their mother.

We have compared the effects of chain-terminating mutations of UBE3A to the effects of missense or amino acid–insertion mutations on clinical characteristics of AS individuals. We have found no significant difference between the two groups with respect to incidence of microcephaly, incidence of seizure disorder, or age of walking independently. This lack of correlation between mutation type and phenotype is not unexpected given that our analysis was limited to individuals with a definite or probable clinical diagnosis of AS. Non–chain-terminating mutations among this class of patients will probably be mutations whose effects on UBE3A function are as severe as those of chain-terminating mutations. Matsuura et al. (1997) described a UBE3A missense mutation (C21Y) transmitted from a normal mother to a child with features milder than those of classical AS, and other missense mutations will probably be detected among patients with similarly mild features.

The detection of UBE3A mutations in some nondeletion/non-UPD/non–imprinting-defect AS individuals and families answers some questions about the pathogenesis of AS and provides the possibility of accurate recurrence-risk counseling and prenatal diagnosis for some families. However, these findings raise many other questions. First, UBE3A mutation analysis increases the percentage of AS individuals in whom one can make a molecular diagnosis from ~75% to ~80%; what is the etiology of AS in the remaining 20%, and what is the recurrence risk? What are the substrates of UBE3A in brain, and what are the biochemical changes in AS brain that lead to the characteristic phenotype? Are the phenotypic abnormalities caused exclusively by complete lack of UBE3A expression in certain brain regions, or is the phenotype also due in part to brain regions where UBE3A expression is reduced but not absent? If AS is caused by failure of ubiquitin-mediated degradation of certain substrates, presumably with accumulation of those substrates, why does the disorder not show a neurodegenerative course, rather than the static course observed? Providing answers to these questions will require continued analysis of UBE3A alterations in AS patients, together with production of animal models in which to test hypotheses generated from human studies.

Acknowledgments

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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 Angelman syndrome gene, UBE3A [U84404])

Online Mendelian inheritance in man (OMIM), http://www.ncbi.nlm.nih.gov/htbin-post/Omim (for Angelman syndrome [MIM 105830])

References

Albrecht U, Sutcliffe JS, Cattanach BM, Beechey CV, Armstrong D, Eichele G, Beaudet AL (1997) Imprinted expression of the murine Angelman syndrome gene, Ube3a, in hippocampal and Purkinje neurons. Nat Genet 17:75–78

Greger V, Knoll JHM, Wagstaff J, Woolf E, Lieske P, Glatt H, Benn PA, et al (1997) Angelman syndrome associated with an inversion of chromosome 15q11.2q24.3. Am J Hum Genet 60:574–580

Huibregtse JM, Scheffner M, Beaudenon S, Howley PM (1995) A family of proteins structurally and functionally related to the E6-AP ubiquitin-protein ligase. Proc Natl Acad Sci USA 92:2563–2567

Huibregtse JM, Scheffner M, Howley PM (1993*a*) Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with p53. Mol Cell Biol 13:775–784

——— (1993*b*) Localization of the E6-AP regions that direct human papillomavirus E6 binding, association with p53, and ubiquitination of associated proteins. Mol Cell Biol 13: 4918–4927

Kishino T, Lalande M, Wagstaff J (1997) UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet 15:70–73

Kishino T, Wagstaff J (1998) Genomic organization of the UBE3A/E6-AP gene and related pseudogenes. Genomics 47: 101–107

Kumar S, Kao WH, Howley PM (1997) Physical interaction between specific E2 and Hect E3 enzymes determines functional cooperativity. J Biol Chem 272:13548–13554

Matsuura T, Sutcliffe JS, Fang P, Galjaard R-J, Jiang Y, Benton CS, Rommens JM, et al (1997) De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nat Genet 15:74–77

Nakao M, Sutcliffe JS, Durtschi B, Mutirangura A, Ledbetter DH, Beaudet AL (1994) Imprinting analysis of three genes in the Prader-Willi/Angelman region: SNRPN, E6-associated

protein, and PAR-2 (D15S225E). Hum Mol Genet 3: 309–315

Rougeulle C, Glatt H, Lalande M (1997) The Angelman syndrome candidate gene, UBE3A/E6-AP, is imprinted in brain. Nat Genet 17:14–15

Vu TH, Hoffman AR (1997) Imprinting of the Angelman syndrome gene, UBE3A, is restricted to brain. Nat Genet 17: 12–13

Wagstaff J, Shugart YY, Lalande M (1993) Linkage analysis in familial Angelman syndrome. Am J Hum Genet 53: 105–112

Williams CA, Zori RT, Hendrickson J, Stalker H, Marum T, Whidden E, Driscoll DJ (1995) Angelman syndrome. Curr Probl Pediatr 25:216–231

Yamamoto Y, Huibregtse JM, Howley PM (1997) The human E6-AP gene (UBE3A) encodes three potential protein isoforms generated by differential splicing. Genomics 41: 263–266