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Transmission of Angelman syndrome by an affected mother

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Purpose: To determine: 1) If a 15q11–13 deletion was transmitted from a female with Angelman syndrome to her fetus, and 2) If the *UBE3A* gene was functionally imprinted in fetal eye. **Methods:** Individuals were genotyped by microsatellite analysis. DNA methylation imprints were assessed by Southern blot analysis and methylation-specific PCR. Expression was analyzed by RT-PCR. **Results:** The mother and fetus inherited large deletions of maternal 15q11–13 and demonstrated paternal-only DNA methylation imprints along 15q11–13. *UBE3A* was paternally expressed in eye tissue from the fetus with Angelman syndrome. **Conclusions:** We show that females with Angelman syndrome are fully capable of reproduction and that *UBE3A* is not imprinted in fetal eye. ***Genetics in Medicine*, 1999:1(6):262–266.**

Key Words: *Angelman syndrome, genomic imprinting, 15q11, 13 deletion, reproduction, DNA methylation*

The Angelman syndrome (AS) is a severe developmental and neurobehavioral disorder characterized by four cardinal features: (1) severe developmental delay; (2) profound speech impairment; (3) a movement and balance disorder, and (4) an AS-specific behavior which includes frequent and inappropriate laughter, a happy affect, and an easily excitable personality.[1,2] Most AS cases arise de novo, the consequences of large deletions from the maternally derived 15q11–13.[2] AS has also been diagnosed in patients with paternal uniparental disomy, imprinting mutations on maternal 15q11–13, and maternally inherited mutations in the 3A ubiquitin protein ligase gene (*UBE3A*).[3] The Prader-Willi syndrome (PWS) is a separate, distinct neurobehavioral syndrome also associated with 15q11–13 deletions. However, the deficiency in PWS is always paternal in origin.[3] All of the cardinal features of AS can be caused by mutations in the *UBE3A* gene,[4,5] which shows imprinted expression in specific regions of the mammalian brain.[6–8]

Individuals with AS achieve puberty normally, with both males and females having normal secondary sexual characteristics, including normal menses in females.[9] However, there has been no documented case of reproduction in either a male or a female with Angelman syndrome. In this report, we present the first documented case of reproduction in an individual with AS. The pregnancy resulted in the maternal transmission of a 15q11–13 deletion to a fetus, which was electively terminated at 16 weeks gestation.

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METHODS

Subjects

The proband is now a 15-year-old female with AS who has been followed intermittently in the Genetics clinic since 22 months of age. Recently, the family recontacted the Genetics service due to the proband's unexpected pregnancy. High-resolution chromosomal analysis in the past demonstrated a 15q11–13 deletion in the proband,[10] which DNA polymorphism and methylation studies have more recently confirmed.

This family was initially reported in 1989,[10] when the concern was raised that the proband's mother, who has normal intelligence, could be mosaic for a submicroscopic deletion of 15q11–13 because she displayed brachycephaly, hearing loss, an enlarged foramen magnum, and mild ataxia. However, extensive cytogenetic and molecular analyses of peripheral blood and skin fibroblasts have failed to reveal any abnormalities in 15q11–13 in the mother of the proband.

The proband has classic AS features[10] with severe mental retardation, an AS-specific behavior, a complete lack of speech, and a movement disorder characterized by ataxia. An abnormal EEG and seizures were also noted at 22 months. She began walking at 3 years, and her severe truncal ataxia has improved with age. The proband also has microbrachycephaly with a head circumference of less than −2 standard deviations, relative prognathism, a protruding tongue, excessive drooling, and an inappropriately happy affect with excessive laughter. Menarche began at 11.5 years. A head CT and MRI were only remarkable for an enlarged foramen magnum.

The family decided to terminate the proband's pregnancy at 15 to 16 weeks gestation. The termination was by suction dilatation and curettage. Identifiable fetal tissue was dissected, immediately frozen in liquid nitrogen, and stored at −70°C. Due to the suction procedure the only intact brain samples obtained were the eyes. Tissue was procured with the approval of

the family and the University of Florida Institutional Review Board.

Microsatellite analysis

DNA was extracted from peripheral blood leukocytes (PBL) and whole tissue according to standard techniques.[11] PCR amplification of the *D15S817*, *D15S128*, *ACTIN* (*ACTC*), *D15S175*, and *D15S87* loci (Research Genetics) was done according to the protocol of Colman et al.[12]

DNA methylation analyses

Southern blot

DNA methylation analyses were performed within 15q11–13 at *SNRPN*[13] and *NECDIN* (*NDN*), and in 11p15 (KvDMR1)[14] according to published protocols. DNA methylation analysis of *NDN* was at a novel *Bss* HII site (AC Lossie, RD Nicholls, DJ Driscoll, unpublished data), which is located within the same *Hin*dIII fragment as the published imprinted *Eag*I site.[15] Specific conditions for each locus are shown in Table 1.

Methylation-specific PCR

Methylation-specific PCR (MSP) relies on the selective ability of sodium bisulfite to convert unmethylated cytosine to uracil, while leaving methylated cytosine unchanged. During PCR amplification, uracil is converted to thymidine, providing a method to distinguish between the methylated and unmethylated alleles. MSP at 5′ *SNRPN* was done according to the manufacturers recommendations (Oncor, Gaithersburg, MD) using primers developed by Kubota and colleagues.[16]

RT-PCR

mRNA was isolated from fetal eye and lymphoblast cell lines using the Polytract System 1000 mRNA extraction kit (Promega, Madison, WI). cDNAs were reverse-transcribed from 20 to 200 ng of mRNA using the Superscript Amplification kit (Gibco, Gaithersburg, MD). RT-PCR analysis of *UBE3A* was accomplished using previously published primers that amplify an 1150 bp fragment from exons 3 to 9 (formerly U1 to 3) of *UBE3A*.[5] A 738 bp fragment of *SNRPN* was amplified using primers specific for exon 2 (5′-TTGGCACACCAGCTGGTACT-3′) through exon 5 (5′-CACCTGAGACGAACTACAG-3′) in a standard reaction with a 57°C annealing temperature. Amplification products were size fractionated by gel electrophoresis, Southern blotted and visualized by autoradiography.

RESULTS

Genetic analysis

The proband, fetus, and parents of the proband were genotyped by microsatellite analysis of loci (see map Fig. 1a) within (*D15S817* and *D15S128*)[17] and distal to 15q11–13 (*ACTIN*, *D15S175*, and *D15S87*). Within 15q11–13, the proband and fetus lacked a maternal genetic contribution (Fig. 1b). At *D15S128*, the proband and fetus only amplified the paternally inherited allele. Although the proband was noninformative at *D15S817*, the fetus showed exclusive amplification of the paternally derived allele. However, the proband and fetus were heterozygous at several loci distal to 15q13, including *D15S175*, *ACTIN* (Fig. 1c), and *D15S87* (data not shown). Therefore, both the proband and fetus have maternally inherited deletions that are restricted to 15q11–13.

DNA methylation analyses

DNA methylation analysis is a powerful diagnostic tool that takes advantage of the differential methylation found in imprinted regions of the genome. The AS/PWS imprinted domain contains several loci that display parent-of-origin methylation imprints.[3] *SNRPN* and *NDN* are two of several paternally expressed genes within 15q11–13 that are preferentially methylated on the repressed maternal allele.[13,15,18]

Two methods can assess DNA methylation at 5′ *SNRPN*: Southern analysis[13,18] and MSP.[16] Southern analysis reveals a 4.3 kb methylated, maternal and a 0.9 kb unmethylated, paternal band in the parents and normal controls. However, the proband and fetus only have the 0.9 kb paternal band (Fig. 2), confirming the lack of a maternal 5′ *SNRPN* allele in these samples. In accordance with Southern analysis, MSP analysis of parental PBL and normal fetal eye DNA shows both the maternal and paternal PCR amplification products. However, PBL DNA from the proband and eye DNA from the fetus exclusively amplified the paternal chromosome 15 (Fig. 3).

Previous methylation studies of the CpG island associated with *NDN* revealed a parent-specific DNA methylation imprint from DNA digested with *Hin*dIII and *Eag*I.[15] A *Bss* HII methylation imprint is also present in this 2.6 kb *Hin*dIII fragment (AC Lossie, RD Nicholls, DJ Driscoll, unpublished data). *NDN* hybridizes to three fragments in control samples: a 2.6 kb maternal-specific, a 1.7 kb constant, and a 1.3 kb paternal-specific fragment (Fig. 4). Normal PBL and fetal eye DNA reveal both the maternal and paternal diagnostic bands. However, PBL DNA from individuals with PWS reveals the

Table 1
Conditions for DNA methylation analysis by Southern blot

GENE	μg DNA	RE	RE	% Gel	V · hrs	Wash (°C)
SNRPN	5	Xba I	Not I	0.8	550	65
NECDIN	10	Hind III	Bss HII	1	1000	70
KVLQT1	5	Eco RI	Not I	1	850	65

Specific restriction enzymes (RE) and Volt · hours (V · hrs) are noted.

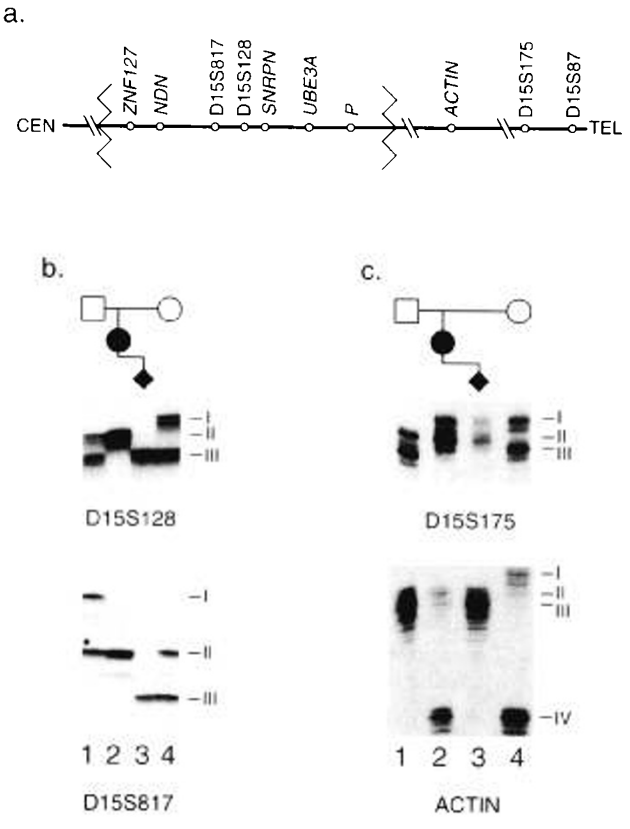

Fig. 1 Microsatellite analysis of chromosome 15. (a) Genetic map of chromosome 15 (not drawn to scale). Common interstitial deletion breakpoints flanking 15q11–13 ({), individual loci (○), centromere (CEN), and telomere (TEL) are noted. (b) Microsatellite analysis of loci within 15q11–13 and (c) Microsatellite analysis of loci distal to 15q11–13. The pedigree shows the father of the proband (lane 1), AS proband (lane 2), proband's fetus (lane 3), and mother of the proband (lane 4). Note the lack of a maternal contribution within 15q11–13 for the proband and fetus at *D15S128*, and for the fetus at *D15S817*. However, distal to 15q11–13, there is a biparental contribution at *ACTIN* and *D15S175*. The father of the AS fetus is unknown.

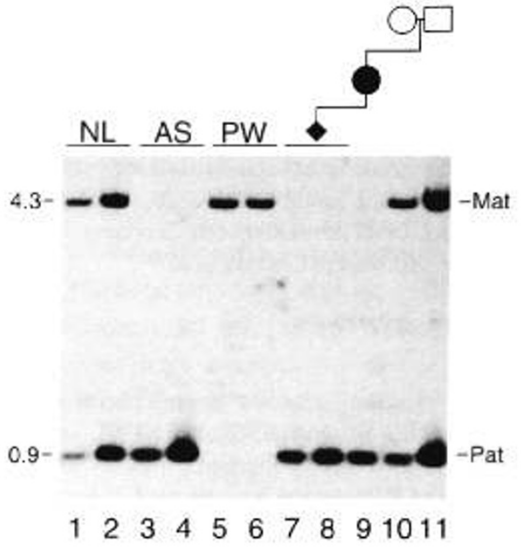

Fig. 2 DNA methylation analysis of 5′ *SNRPN*. Digestion of DNA with *XbaI* and *NotI* reveals diagnostic bands of 4.3 kb (methylated, maternal) and 0.9 kb (unmethylated, paternal). PBL DNA from normal individuals (NL) reveals both the 4.3 kb and 0.9 kb normal methylation profile (lanes 1, 2, 10, 11). Patients with AS only have the 0.9 kb unmethylated fragments (lanes 3 and 4), while individuals with PWS have the 4.3 kb methylated allele (lanes 5 and 6). Eye DNA from the fetus (lanes 7 and 8) and PBL DNA from the AS proband (lane 9) reveal AS-specific DNA methylation imprints, while PBL DNA from the parents shows maternal- and paternal-specific bands (lanes 10 and 11).

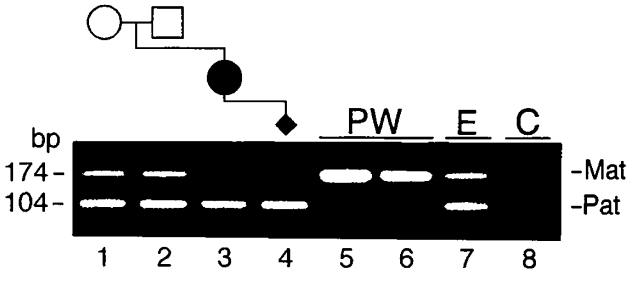

Fig. 3 MSP analysis of 5′ *SNRPN*. PBL (lanes 1, 2, 3, 5, and 6) and fetal eye DNAs (lanes 4 and 7) are shown. The pedigree shows the positions of each family member. Normal individuals (lanes 1, 2, and 7) amplified the 174 bp methylated and 104 bp unmethylated products. The proband (AS) and fetus (AS) exclusively amplified the unmethylated allele, while two patients with PWS (lanes 5 and 6) amplified only the methylated allele. PW (Prader-Willi), normal fetal eye (E), and water control (C) are indicated.

predominance of the methylated 2.6 kb fragment, while *NDN* hybridized to the paternal-specific unmethylated 1.3 kb fragment in patients with AS. DNA from the parents of the AS proband demonstrates normal biparental methylation, while AS methylation imprints are apparent in PBL DNA from the proband with AS and eye tissue from the fetus with AS.

A differentially methylated region (KvDMR1) near an antisense transcript of the *KvLQT1* gene[14] in the Beckwith-Weideman syndrome region on chromosome 11 was also examined. All of our control and family samples showed normal DNA methylation imprints at this nonchromosome 15 differentially methylated site (data not shown).

Gene expression

RT-PCR analyses of AS fetal eye, normal fetal eye, and PWS lymphoblast cells revealed a 1.4 kb transcript from exons 3 to 9 of *UBE3A* in all tissues examined (Fig. 5). However, as expected, *SNRPN* was only expressed in the AS and normal tissues; the PWS sample failed to amplify *SNRPN*.[13]

DISCUSSION

The presence of AS-specific DNA methylation imprints and absence of a maternal contribution of proximal 15q demonstrates that the patient with AS passed her 15q11–13 deletion to her fetus. These findings conclusively demonstrate that females with AS are capable of reproduction and show that the previously observed lack of reproduction is most likely due to social/cognitive behavior rather than physiologic reasons. Therefore, appropriate appraisal of the reproductive risks should be part of the genetic counseling given to AS families.

Recent studies by Gabriel and colleagues[19] of a mouse deletion model for the AS/PWS imprinted region support our findings. Their model, a transgene insertion/deletion ($Tg^{PWS/AS(del)}$) of the syntenic region on mouse chromo-

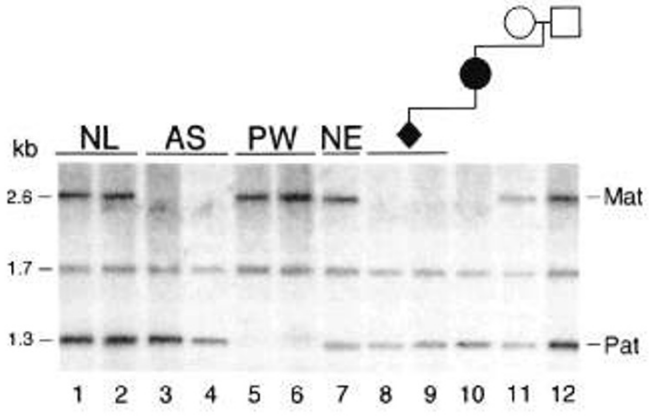

Fig. 4 DNA methylation analysis of *NDN*. Digestion of DNA with *Bss* HII and *Hin*dIII reveals diagnostic bands of 2.6 kb (methylated, maternal) and 1.3 kb (unmethylated, paternal), and a constant band of 1.7 kb. PBL DNA from normal individuals (lanes 1, 2, 11, 12) and normal fetal eye (NE; lane 7) shows the normal DNA methylation profile. Patients with AS (lanes 3 and 4) have the 1.3 kb paternal diagnostic band, while individuals with PWS (lanes 5 and 6) predominantly have the 2.6 kb methylated, maternal fragment. Eye DNA from the fetus and PBL DNA from the proband reveal AS-specific DNA methylation imprints (lanes 8, 9, and 10).

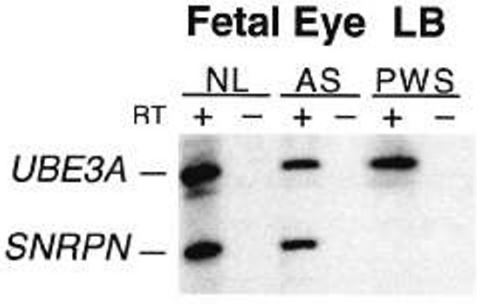

Fig. 5 Expression analysis of *UBE3A* and *SNRPN*. A Southern blot of RT-PCR products from *UBE3A* exons 3 to 9 (1150 bp) and *SNRPN* exons 2 to 5 (738 bp) is shown. The membrane was hybridized with the same RT-PCR products. Reactions with (+) and without (−) RT are noted. *UBE3A* and *SNRPN* transcripts are present in normal (lane 1) and AS fetal eye (lane 3). However, only *UBE3A* is expressed in lymphoblast cells from a patient with PWS (lane 5). Extra bands are due to alternative splicing of *UBE3A*.

some 7C, correlates well with our findings in this patient with AS, as a large murine chromosomal deletion can also be passed through the maternal germline. Mice that inherit the maternal deletion have many of the characteristics found in individuals with Angelman syndrome.

The ability of an AS mouse to pass on either a normal or deleted chromosome 7C allows a closer examination of the role that replication timing[20] and homologous association[21] play in the establishment and transmission of the chromosome 15q11–13 imprint. In the mouse model for AS, maternal transmission of a large deletion encompassing the imprinted domain failed to interrupt the proper imprint establishment of the normal AS/PWS chromosomal region.[19] These findings demonstrate that during murine gametogenesis, the imprinting on both homologues must be established and transmitted in *cis*, and that neither replication timing nor homologous association are required for the establishment or transmission of the imprint. In addition, these data strongly suggest that inheritance of a normal chromosome 15 from a mother with AS would result in a normal offspring.

Evidence for imprinted expression of the human *UBE3A* gene is limited. Murine studies demonstrate that imprinted expression of *Ube3a* is limited to the CA2 and CA3 regions of the hippocampus, the mitral cell layer of the olfactory bulb, and Purkinje cells of the cerebellum.[6] In humans, evidence is less precise. Imprinted expression of *UBE3A* was observed in adult frontal cortex[7] and fetal brain (unspecified).[8] Because the eyes originate from the lateral aspects of the forebrain during fetal development, it was possible that fetal eye would also show imprinted expression. However no difference in the expression of *UBE3A* was observed between normal and AS fetal eye samples.

Imprinted genes in other chromosomal regions also show DNA methylation imprints.[22] To determine if the potential interruption of the imprinting process in the AS/PWS region could alter DNA methylation imprints at loci on 11p15, we examined KvDMR1, a differentially methylated site associated with an antisense transcript of *KvLQT1*.[14] All samples showed normal DNA methylation, indicating that the establishment and transmission of this 11p15 DNA methylation imprint occurs independently from the AS/PWS region.

In conclusion, our findings establish that the Angelman syndrome can be transmitted through a female with AS, imply that the Prader-Willi syndrome could also be transmitted through an AS deletion male, and illustrate the need for informed reproductive counseling for parents of individuals with AS

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