RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Angelman Syndrome: Mutations Influence Features in Early Childhood

Wen-Hann Tan,[1,2] Carlos A. Bacino,[1,3] Steven A. Skinner,[1,4] Irina Anselm,[1,5] Rene Barbieri-Welge,[1,6] Astrid Bauer-Carlin,[1,4] Arthur L. Beaudet,[1,3] Terry Jo Bichell,[1,7] Jennifer K. Gentile,[1,8] Daniel G. Glaze,[1,9] Lucia T. Horowitz,[1,4] Sanjeev V. Kothare,[1,5] Hye-Seung Lee,[1,10] Mark P. Nespeca,[1,11] Sarika U. Peters,[1,12] Trilochan Sahoo,[1,13] Dean Sarco,[1,5] Susan E. Waisbren,[1,8] and Lynne M. Bird[1,14]*

[1]NIH Rare Diseases Clinical Research Network—Angelman, Rett, & Prader-Willi Syndromes Consortium

[2]Division of Genetics, Children's Hospital Boston; Harvard Medical School, Boston, Massachusetts

[3]Kleberg Genetics Clinic, Texas Children's Hospital; Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas

[4]Greenwood Genetic Center, Greenwood, South Carolina

[5]Department of Neurology, Children's Hospital Boston; Harvard Medical School, Boston, Massachusetts

[6]Developmental Services, Rady Children's Hospital San Diego, San Diego, California

[7]Vanderbilt Kennedy Center, Vanderbilt University, Nashville, Tennessee

[8]Department of Psychiatry, Children's Hospital Boston; Harvard Medical School, Boston, Massachusetts

[9]Texas Children's Hospital; Section of Neurology, Department of Pediatrics, Baylor College of Medicine, Houston, Texas

[10]Pediatric Epidemiology Center, Department of Pediatrics, University of South Florida, Tampa, Florida

[11]Division of Neurology, Rady Children's Hospital San Diego, Department of Neuroscience, University of California, San Diego, California

[12]Meyer Center for Developmental Pediatrics, Texas Children's Hospital; Section of Developmental Pediatrics, Baylor College of Medicine, Houston, Texas

[13]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas

[14]Division of Genetics/Dysmorphology, Rady Children's Hospital San Diego; Department of Pediatrics, University of California, San Diego, California

Received 16 January 2010; Accepted 11 September 2010

Angelman syndrome (AS) is a neurodevelopmental disorder caused by a lack of expression of the maternal copy of *UBE3A*. Although the "classic" features of AS are well described, few large-scale studies have delineated the clinical features in AS. We present baseline data from 92 children with a molecular diagnosis of AS between 5 and 60 months old who are enrolled in the National Institutes of Health Rare Diseases Clinical Research Network Angelman Syndrome Natural History Study from January 2006 to March 2008. Seventy-four percent of participants had deletions, 14% had either uniparental disomy (UPD) or imprinting defects, and 12% had *UBE3A* mutations. Participants with UPD/imprinting defects were heavier ($P = 0.0002$), while those with deletions were lighter, than the general population ($P < 0.0001$). Twenty out of 92 participants were underweight, all of whom had deletions or *UBE3A* mutations. Eight out of 92 participants (6/13 (46%) with UPD/imprinting defects and 2/11 (18%) with *UBE3A* mutations) were obese. Seventy-four out of 92 participants (80%) had absolute or relative microcephaly. No participant was macrocephalic. The most common behavioral findings were mouthing behavior (95%), short attention span (92%), ataxic or broad-based gait (88%), history of sleep difficulties (80%), and fascination with water

Grant sponsors: National Center for Research Resources (NCRR) and Office of Rare Diseases Research (ORDR), National Institutes of Health (NIH); Grant numbers: NIH U54 RR019478 and NIH U54 RR019259; Grant sponsor: Angelman Syndrome Foundation—Western Area Chapter.

Current address of Sarika U. Peters is Vanderbilt Kennedy Center, Vanderbilt University, Nashville, TN.

Current address of Trilochan Sahoo is Signature Genomic Laboratories, LLC, Spokane, WA.

Current address of Dean Sarco is Neurology Department, Southern California Permanente Medical Group, Los Angeles, CA.

*Correspondence to:
Lynne M. Bird, M.D., 3020 Children's Way #5031, San Diego, CA 92123.
E-mail: lbird@rchsd.org

Published online 22 December 2010 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.33775

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(75%). Frequent, easily provoked laughter was observed in 60%. Clinical seizures were reported in 65% of participants but all electroencephalograms (EEGs) were abnormal. We conclude that the most characteristic feature of AS is the neurobehavioral phenotype, but specific EEG findings are highly sensitive for AS. Obesity is common among those with UPD/imprinting defects.
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Key words: Angelman syndrome; genotype–phenotype correlation; behavioral genetics; growth; child development

How to Cite this Article:
Tan W-H, Bacino CA, Skinner SA, Anselm I, Barbieri-Welge R, Bauer-Carlin A, Beaudet AL, Bichell TJ, Gentile JK, Glaze DG, Horowitz LT, Kothare SV, Lee H-S, Nespeca MP, Peters SU, Sahoo T, Sarco D, Waisbren SE, Bird LM. 2011. Angelman syndrome: Mutations influence features in early childhood.
Am J Med Genet Part A 155:81–90.

INTRODUCTION

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by intellectual disability (mental retardation), limited speech, and behavioral features such as a happy disposition, easily provoked laughter, mouthing of objects, and fascination with water [Angelman, 1965; Buntinx et al., 1995; Clayton-Smith and Laan, 2003; Williams et al., 2006]. The prevalence of AS is approximately 1:15,000 although it is probably under-diagnosed [Kyllerman, 1995; Petersen et al., 1995; Buckley et al., 1998]. AS is caused by a lack of expression of the maternal copy of *UBE3A* (ubiquitin protein ligase E3A) in the brain where it is imprinted such that the paternal copy of *UBE3A* is normally silenced [Albrecht et al., 1997; Kishino et al., 1997; Matsuura et al., 1997; Rougeulle et al., 1997; Vu and Hoffman, 1997]. There are four known molecular mechanisms that lead to the deficiency of maternal *UBE3A* expression: deletion of the AS critical region on maternal chromosome 15q11–q13, paternal uniparental disomy (UPD), imprinting defects leading to a lack of maternal imprint and expression of *UBE3A*, and mutations in the maternal copy of *UBE3A*. Previous studies have suggested that approximately 70% of AS individuals have deletions, 2–7% have paternal UPD, 3–5% have imprinting defects, and about 10% have mutations in maternal *UBE3A* [Williams et al., 1993–2009; Clayton-Smith and Laan, 2003]. Approximately 10% of individuals with a clinical diagnosis of AS have either unidentifiable molecular defects [Williams et al., 1993–2009; Lossie et al., 2001], or other conditions that resemble AS [Williams et al., 2001; Gilfillan et al., 2008; Zweier et al., 2008]. Deletions are typically 5.9 Mb (class I) or 5.0 Mb (class II) in size, differing only by the location of the centromeric breakpoint [Knoll et al., 1990; Christian et al., 1995]; atypical deletions that are larger or smaller than these two common deletions have also been reported [Sahoo et al., 2006, 2007]. About 10–20% of imprinting defects are due to deletions in the AS imprinting center; the remainder result from a presumed failure in "re-programming" during oogenesis or in the embryo (i.e., epimutations) [Williams et al., 1993–2009; Buiting et al., 2003].

Although clinical diagnostic criteria for AS have been established [Williams et al., 2006], few large-scale studies on the clinical features of AS have been conducted [Clayton-Smith, 1993; Saitoh et al., 1994; Lossie et al., 2001], and the natural history remains incompletely characterized. The prevalence of the various clinical features of AS depends on the molecular etiology; individuals with deletions on chromosome 15 are generally more severely affected than individuals with AS due to other etiologies [Burger et al., 1996; Moncla et al., 1999b; Lossie et al., 2001; Varela et al., 2004], and some small studies have shown an association between deletion sizes and phenotypic features [Varela et al., 2004; Sahoo et al., 2006, 2007].

To improve our understanding of the natural history of AS, a 5-year multi-center longitudinal study was initiated. Here we present the baseline data from all participants up to the age of 5 years who were enrolled in the first $2^1/_4$ years of the study to provide an overview of the common clinical features of AS and the correlations with genotypes among the younger children. The purpose of this report is to characterize the clinical features of AS in young children in order to help primary care providers and pediatric subspecialists recognize AS more readily, especially in young children who may not exhibit the "classical" clinical picture.

PARTICIPANTS AND METHODS

Participants

All participants were enrolled between January 2006 and March 2008 in the Angelman Syndrome Natural History Study (ClinicalTrials.gov Identifier: NCT00296764) that is being conducted by the Angelman, Rett, and Prader–Willi Syndromes Consortium of the NIH Rare Diseases Clinical Research Network (RDCRN) at one of four study sites—Rady Children's Hospital San Diego, Texas Children's Hospital, Greenwood Genetic Center, and Children's Hospital Boston. The inclusion criteria were: (i) a molecular diagnosis of AS, or a clinical diagnosis based on the criteria specified in Table I, and (ii) age between 1 day and 60 years old. The exclusion criteria were: (i) presence of a co-morbid disorder that is not a known feature of AS (e.g., inborn errors of metabolism, brain trauma) and (ii) birth before 28 weeks of gestation.

Participants were recruited through parent support groups and referrals from professional colleagues. Parents and healthcare professionals could contact the investigators through the public website, http://ClinicalTrials.gov/. The Institutional Review Board at each study site and the Data and Safety Monitoring Board of the RDCRN approved this study. The legal guardian of each participant provided written informed consent.

TABLE I. Clinical Diagnostic Criteria for AS

Major criteria (all required)
Developmental Delay, functionally severe
Speech impairment (no words or minimal words used)
Movement or balance disorder
Behavioral characteristics (frequent or easily provoked laughter/smiling, excitable personality, hand flapping, short attention span)
Minor criteria: (at least three required for eligibility)
Postnatal deceleration in head growth
Seizures
Abnormal electroencephalogram (with patterns suggestive of AS, or hypsarrythmia)
Sleep disturbance
Attraction to, or fascination with, water
Drooling

AS, Angelman syndrome.

Clinical Evaluation of Participants

Each participant was evaluated by a clinical geneticist. A structured medical history and a physical examination were performed. Specific behavioral characteristics were elicited from the caregivers through questions requiring a dichotomous response (''yes,'' ''no,'' or ''unknown''). The caregivers were also asked whether the participants' skin and hair colors were lighter than expected. Neurodevelopmental assessments were performed by child psychologists using the Bayley Scales of Infant and Toddler Development, Third Edition (Bayley-III) [Bayley, 2005], and the Vineland Adaptive Behavior Scales, Second Edition (VABS-II) [Sparrow et al., 2005]. The Bayley-III Cognitive domain provides an overall measure of cognition based on a set of standardized tasks that the participant is asked to perform, while the VABS-II Adaptive Behavior Composite assesses the abilities of the participant in his or her environment based on a questionnaire administered to the parents/caregivers. As such, the Bayley-III provides an objective measure of a participant's ability as assessed by a psychologist on one occasion, whereas the VABS-II scores depend on (subjective) parental observations but allow for the repeated assessment of the participant's level of functioning in a more ''natural'' (home) environment. Children with developmental disabilities should be assessed using instruments that are appropriate for their developmental age rather than chronological age [Lichtenberger, 2005], and since previous studies have suggested that AS children are very unlikely to achieve a developmental age beyond 42 months in any of the Bayley-III developmental domains, we decided that this was an appropriate developmental instrument for our cohort. However, since the Bayley-III standard scores are only normed through 42 months of age and many children with severe developmental disabilities score at the floor of this test, the Bayley-III standard scores do not yield meaningful comparisons of functional skills among participants. Instead, we calculated the developmental quotient (DQ) by dividing the developmental age by the chronological age and multiplying by 100, which also controls for the effects of chronological age and differences in age among different molecular subtypes. On the other hand, the VABS-II has been validated for individuals with intellectual disability through age 90 years, hence standard scores (mean: 100, standard deviation: 15) were compared between the different AS molecular types. These standard scores are independent of chronological age and can be considered a measure of developmental functioning.

Electroencephalogram (EEG)

Clinical 16–18 channel electroencephalogram (EEG) studies were performed on participants with a full complement of electrodes placed using the standard 10/20 system. Recordings of at least 30 min in both awake and asleep states were attempted without sedation, although some participants had only awake or asleep EEGs. All studies were reviewed by board-certified clinical neurophysiologists.

Determination of Size of Chromosome 15 Deletion

DNA was extracted from peripheral white blood cells of all participants with deletions on chromosome 15. Deletion breakpoints on chromosome 15q11.2–q14 were determined by microarray-based comparative genomic hybridization utilizing a chromosome 15-specific bacterial artificial chromosome (BAC) or oligonucleotide array. The detailed methodologies have been previously described [Sahoo et al., 2005, 2008]. Deletion sizes were based on the positions of the first and the last BAC clones or oligonucleotide probes within the region of the deletion that showed a heterozygous loss in copy number.

Statistical Analyses

Clinical outcomes of interest were summarized for the entire cohort. For continuous outcomes, the median, 5th and 95th centiles were reported and analyzed using the Kruskal–Wallis test for comparisons across the three molecular subclasses (Deletions, Imprinting defects/UPD, and *UBE3A* mutations), and the Mann–Whitney test for comparisons between deletion classes I and II, because our data did not necessarily fit the Normal distribution. For categorical outcomes, the proportions were compared using either chi-square or the Fisher's exact test, depending on the frequency in each cell. Confidence intervals (CI) for proportions were calculated by the exact binomial (Clopper–Pearson) method.

TABLE II. Age, Gender, and Molecular Subclass Distribution Among Participants

	Total	Deletion class I	Deletion class II	Deletion (other)	All deletions	UPD/imprinting defects	*UBE3A* mutation
Age (months)							
0–24	27	12	9	4	25	0	2
25–36	26	5	13	0	18	5	3
37–60	39	12	13	0	25	8	6
Total	92	29 (32%)	35 (38%)	4 (4%)	68 (74%)	13 (14%)	11 (12%)
Gender							
M	50	13	22	0	35	8	7
F	42	16	13	4	33	5	4

UPD, uniparental disomy; M, Male; F, Female.
Also published in Gentile et al., [2010].

The gestational age-corrected *z*-scores for birth and growth parameters were calculated using the United Kingdom 1990 reference curves [Cole et al., 1998]. These reference curves were used instead of those published by the United States Centers for Disease Control and Prevention (CDC) because the CDC reference curves do not include head circumference data for individuals older than 36 months. Comparisons with the general population were made using the signed-rank test with an expected median of 0.

RESULTS

General Characteristics

There are 92 participants in this report, all of whom had a molecular diagnosis of AS. The median age was $33^1/_2$ months (range: 5–60 months). Table II shows the frequency of molecular subclasses and the distribution of the various age groups. Participants with UPD (n = 5), imprinting defects (n = 7), and abnormal methylation but without a deletion and had no further molecular studies (n = 1) were grouped together (collectively known as "UPD/imprinting defects"). These subclasses were analyzed together as a single entity because each of these subclasses had too few participants for any meaningful statistical comparisons with the deletion and the *UBE3A* mutation subclasses. Moreover, those with UPD and imprinting defects are expected to be more closely related to each other from a genetic perspective than they are to the other subclasses because in these two subclasses, both copies of all the genes in the AS critical region are present. In contrast, the deletion participants are haploinsufficient for all the genes in this region, while in the *UBE3A* mutation participants, the maternal copy of *UBE3A* is the only non-functional gene in the critical region. The "Deletion (other)" category included participants with atypical (neither class I nor class II) deletions. Participants with deletions were significantly younger (median age: $28^1/_2$ months) than the "non-deletion" participants as a group (median age: 40 months) ($P = 0.028$). There was no significant difference in the ages between those with class I and those with class II deletions ($P = 0.71$).

Fourteen participants, all of whom had AS due to a deletion, were suspected of having AS by either a general pediatrician (n = 11) or a parent (n = 3); in the remaining 78 participants, the diagnosis was first suspected by a pediatric subspecialist (geneticist (n = 47), neurologist or neuropediatrician (n = 28), other pediatric subspecialists (n = 3)). The median age at diagnosis among all participants was 16 months (range: 1–39 months); 95% were diagnosed before 36 months old. Participants with UPD, imprinting defects, and *UBE3A* mutations were diagnosed later (median age: 24 months) compared to those with deletions (median age: 14 months) ($P < 0.001$), suggesting that the non-deletion population may present in ways that are less well recognized. There was no significant difference in the age of diagnosis between participants with classes I and II deletions ($P = 0.91$).

Growth

The gestational age-adjusted *z*-scores of the birth and postnatal growth parameters, where available, were calculated for the entire cohort and for each molecular subclass (Table III). At the time of evaluation, participants with UPD/imprinting defects were significantly heavier than the general population ($P = 0.0002$), while those with deletions (particularly class I deletions) were significantly lighter than the general population ($P < 0.0001$). Comparing molecular subclasses, participants with class I deletions were the lightest and shortest, and had the lowest body mass index (BMI), while those with UPD/imprinting defects were the tallest, heaviest, and had the highest BMI, although the differences in the heights among the participants in the different molecular subclasses were not statistically significant. Of the 20/92 (22%) participants who were underweight (BMI < 5th centile), all had either deletions or *UBE3A* mutations. Nine percent (8/92) of participants were obese (BMI > 95th centile), including six (46%) of those with UPD/imprinting defects, and two (18%) of those with *UBE3A* mutations; none of the participants with deletions were obese. Among participants with UPD/imprinting defects, none had a BMI below the 84th centile.

Absolute or relative microcephaly (i.e., head circumference more than 2 SD below the mean, or *z*-score of head circumference more than 2 SD below *z*-score of length) was observed in 74/92 (80%) of participants. The prevalence of microcephaly varied by molecular etiology: 58/68 (85%) in participants with a deletion, 8/13 (62%) in those with UPD/imprinting defects, and 8/11 (73%) in those with *UBE3A* mutations. As expected, no participant was macrocephalic (head circumference more than 2 SD above the mean).

TABLE III. Z-Scores for Growth Parameters: Median (5th, 95th Centiles); n = Number of Participants

	Entire cohort	Deletions			UPD/imprinting defects	*UBE3A*
		Class I	Class II	All		
Birth weight	0.24 (−1.42, 1.55) (n = 91)	0.10 (−1.90, 0.79) (n = 29)	0.23 (−1.47, 2.25) (n = 34)	0.18 (−1.66, 1.42) (n = 67)	0.50 (−0.56, 2.14) (n = 13)	0.23 (−1.11, 0.59) (n = 11)
Birth length	0.55 (−1.74, 2.58) (n = 73)	−0.02 (−2.41, 2.51) (n = 21)	0.54 (−1.97, 2.55) (n = 26)	0.31 (−2.32, 2.55) (n = 50)	1.06 (0.08, 2.85) (n = 12)	−0.32 (−1.18, 2.09) (n = 11)
Weight	−0.27 (−2.50, 2.17) (n = 92)	−0.91* (−2.59, 0.34) (n = 29)	−0.45 (−2.17, 1.38) (n = 35)	−0.53* (−2.58, 1.08) (n = 68)	1.61* (0.64, 3.16) (n = 13)	−0.20 (−1.72, 2.12) (n = 11)
Length or Height	0.04 (−1.76, 1.84) (n = 92)	−0.18 (−1.82, 0.73) (n = 29)	0.20 (−1.76, 1.75) (n = 35)	−0.01 (−1.90, 1.48) (n = 68)	0.19 (−0.84, 2.87) (n = 13)	−0.06 (−1.11, 1.77) (n = 11)
Body mass index	−0.35 (−2.97, 2.14) (n = 92)	−0.63 (−3.35, 0.69) (n = 29)	−0.61 (−2.65, 0.99) (n = 35)	−0.60* (−3.18, 0.84) (n = 68)	1.36* (1.06, 3.03) (n = 13)	0.28 (−1.93, 2.26) (n = 11)
Head circumference	−2.59* (−4.63, −0.61) (n = 92)	−3.05* (−4.50, −1.46) (n = 29)	−2.83* (−4.64, −1.49) (n = 35)	−2.85* (−4.66, −1.46) (n = 68)	−1.41* (−2.61, −0.43) (n = 13)	−1.99 (−3.46, −0.10) (n = 11)

UPD, uniparental disomy.

[a] $P < 0.001$ compared to the general population (i.e., median z-score of 0).

Neurological and Developmental Characteristics in AS

Ataxic or broad-based gait was observed in 36/41 (88%) of all ambulatory participants, being more common among participants with deletions than those with UPD/imprinting defects, although the difference was not statistically significant (Table IV). Thirteen participants (five with deletions, five with UPD/imprinting defects, and three with *UBE3A* mutations) walked with arms upheld and flexed at the elbows. Only 3/41 ambulatory participants (two with imprinting defects, one with *UBE3A* mutation) had a normal gait.

Although EEG abnormalities were recorded in all 84 participants who had an EEG, only 65% (95% CI: 55–75%) of all participants have ever had clinical seizures. Seizures were reported in 83% of participants with a class I deletion, but in only 55% of those with *UBE3A* mutations and 46% of those with UPD/imprinting defects. The median age among the participants who have had at least one clinical seizure was 38 months (range: 11–60 months), while the median age among those who have never had seizures was 28 months (range: 5–59 months) ($P = 0.01$). Among those who have had seizures, the median age of onset of seizures was $15^1/_2$ months old (range: 22 hr of life to 53 months old), with 95% occurring by 36 months of age. The seizure types included generalized (absence and tonic-clonic) and partial complex seizures. EEG abnormalities included background slowing, very high voltage slow delta activity, and focal, multifocal, or generalized epileptiform abnormalities.

The neurodevelopmental profile has been reported in detail elsewhere [Gentile et al., 2010 A neurodevelopmental survey of AS with genotype–phenotype correlations. *J Dev Behav Pediatr* 2010; In press]. Compared to the non-deletion participants, those with deletions had lower cognitive scale DQs on the Bayley-III ($P = 0.0047$) and lower adaptive behavior composite on the VABS-II ($P = 0.014$). There were no significant differences between participants with deletion class I and those with deletion class II ($P = 0.74$ for Bayley-III, $P = 0.90$ for VABS-II) on these two neurodevelopmental measures.

Other Clinical and Behavioral Features Observed in AS

The behavioral phenotype of AS was most typified by mouthing behavior, short attention span, and fascination with water, especially in participants with deletions (Table V). Although these individuals have a generally happy disposition, easily provoked laughter was observed in fewer than 65% across all molecular subclasses. Sleep difficulties (i.e., reduced need for sleep or frequent night-time waking), were seen in at least 73% of the participants. Mouthing behaviors tended to be more common among participants with deletions compared to the non-deletion participants ($P = 0.016$). Participants with deletions also tended to have lighter hair and skin color than the non-deletion participants ($P = 0.021$). There were no other statistically significant differences in the prevalence of the features listed in Table V among participants with deletions, UPD/imprinting defects, and *UBE3A* mutations.

TABLE IV. Neurological Features and Cognitive Measures: Frequency (%) [95% Confidence Interval] or Median (5th, 95th centiles)

		Deletions				
	Entire cohort	Class I	Class II	All	UPD/imprinting defects	*UBE3A* mutation
Ataxic or broad-based gait	36/41 (88%) [74–96%]	9/10 (90%) [56–99.8%]	12/12 (100%) [74–100%]	21/22 (95%) [77–99.9%]	8/11 (73%) [39–94%]	7/8 (88%) [47–99.7%]
Clinical seizures	60/92 (65%) [55–75%]	24/29 (83%) [64–94%]	22/35 (63%) [45–79%]	48/68 (71%) [58–81%]	6/13 (46%) [19–75%]	6/11 (55%) [23–83%]
Age of seizure onset (months) [median; 5th, 95th centile]	16 (4, 36)	14 (6, 36)	22 (12, 35)	18 (6, 36)	7 (1, 33)	11 (4, 21)
Floppy infant (by history)	46/91 (51%) [40–61%]	14/29 (48%) [29–67%]	20/35 (57%) [39–74%]	38/68 (56%) [43–68%]	6/12 (50%) [21–79%]	2/11 (18%) [2–52%]
Hypotonia (trunk/limbs) at evaluation	29/89 (33%) [23–43%]	13/28 (46%) [28–66%]	9/33 (27%) [13–46%]	26/65 (40%) [28–53%]	0/13 (0%) [0–25%]	3/11 (27%) [6–61%]
Hypertonia (limbs) at evaluation	20/89 (22%) [14–33%]	8/28 (29%) [13–49%]	11/33 (33%) [18–52%]	19/65 (29%) [19–42%]	1/13 (8%) [0.1–36%]	0/11 (0%) [0–28%]
Normal tone at evaluation	44/89 (49%) [39–60%]	8/28 (29%) [13–49%]	16/33 (48%) [31–66%]	24/65 (37%) [25–50%]	12/13 (92%) [64–99.8%]	8/11 (73%) [39–94%]
VABS-II Adaptive Behavior Composite Standard score [median; 5th, 95th centile]	61 (48, 75)	60 (47, 70)	60 (48, 73)	60 (47, 71)	61 (57, 67)	66 (55, 87)

UPD, uniparental disomy; VABS-II, Vineland Adaptive Behavior Scales, Second Edition.

DISCUSSION

As part of an ongoing natural history study, we present the common phenotypic features observed in our initial evaluation of 92 children with AS up to 5 years old. Although molecular diagnosis of AS is not required for participation if the clinical diagnostic criteria (Table I) are met, all participants in this report had molecularly proven AS. The distribution of the molecular etiology among our participants is consistent with previous studies [Clayton-Smith and Laan, 2003; Williams et al., 1993–2009]. Therefore, we believe that our data are representative of the clinical manifestations of AS in children up to 5 years old. Although this is one of the largest cohorts of AS individuals in the medical literature, we have relatively few participants with UPD/imprinting defects and *UBE3A* mutations, and hence the results in these two subclasses should be interpreted with caution.

Nearly half of the children with UPD/imprinting defects in our study were obese and all but one were overweight (BMI > 85th centile) despite a high prevalence of feeding difficulties in infancy. Obesity has previously been reported in AS, particularly among the "non-deletion" AS individuals, but it is generally thought that obesity occurs mainly in older individuals [Williams et al., 2006]. Several children in our study with UPD/imprinting defects were reported to have food-seeking behavior reminiscent of children with Prader–Willi syndrome, which raises the question of why having two presumably epigenetically identical copies of chromosome 15 results in food-seeking behavior. Having two copies of chromosome 15 with a paternal imprint might lead to over-expression of the paternally expressed genes on that chromosome. However, in Prader–Willi syndrome, there is a lack of expression of these paternally expressed genes. Therefore, it is difficult to reconcile how any of the paternally expressed genes on chromosome 15 could be directly responsible for the hyperphagia and obesity seen in AS individuals with UPD/imprinting defects.

In contrast to the previously reported studies, only 71% of our participants with deletions and fewer than 60% of our participants with either UPD/imprinting defects or *UBE3A* mutations had clinical seizures. However, our study may have underestimated the prevalence of seizures, especially in those with UPD/imprinting defects in whom the age of seizure onset is usually between 5 and 6 years old [Moncla et al., 1999b; Lossie et al., 2001; Varela et al., 2004]. On the other hand, 95% of the participants with deletions who had experienced seizures did so by the age of 36 months, which suggests that our estimated prevalence of seizures in this subgroup of AS children may be reasonably valid. Nonetheless, abnormal EEG findings similar to those previously reported in this population such as rhythmic 4–6 Hz activity that persists with eye closure and high voltage slow 2–3 Hz delta activity with spikes and sharp waves were observed in all 84 participants who had this investigation [Laan and Vein, 2005]. This suggests that specific EEG findings may provide clues to the diagnosis of AS even in the very young children who have not had any clinical seizures, and it may be an effective screening tool with what appears to be 100% sensitivity, even if not highly specific for AS.

Frequent, inappropriate, or easily provoked laughter is another defining feature of AS [Angelman, 1965]. This has previously been observed in 96–97% of individuals with AS due to a deletion [Saitoh

TABLE V. Clinical Features and Behavioral Traits: Frequency (%) [95% Confidence Interval]

		Deletions				
	Entire cohort	**Class I**	**Class II**	**All**	**UPD/imprinting defects**	***UBE3A***
Feeding difficulties in infancy	59/90 (66%) [55–75%]	22/29 (76%) [56–90%]	19/35 (54%) [37–71%]	43/67 (64%) [52–76%]	10/13 (77%) [46–95%]	6/10 (60%) [26–88%]
Gastroesophageal reflux disease	65/92 (71%) [60–80%]	23/29 (79%) [60–92%]	20/35 (57%) [39–74%]	46/68 (68%) [55–78%]	9/13 (69%) [39–91%]	10/11 (91%) [59%–99.8%]
Drooling	68/92 (74%) [64–83%]	23/29 (79%) [60–92%]	25/35 (71%) [54–85%]	50/68 (74%) [61–83%]	10/13 (77%) [46–95%]	8/11 (73%) [39–94%]
Mouthing behavior	87/92 (95%) [88–98%]	28/29 (97%) [82–99.9%]	35/35 (100%) [90–100%]	67/68 (99%) [92–99.96%]	11/13 (85%) [55–98%]	9/11 (82%) [48–98%]
History of sleep difficulties	74/92 (80%) [71–88%]	23/29 (79%) [60–92%]	27/35 (77%) [60–90%]	54/68 (79%) [68–88%]	12/13 (92%) [64–99.8%]	8/11 (73%) [39–94%]
Fascination with water	69/92 (75%) [65–83%]	25/29 (86%) [68–96%]	28/35 (80%) [63–92%]	54/68 (79%) [68–88%]	8/13 (62%) [32–86%]	7/11 (64%) [31–89%]
Hand flapping	58/92 (63%) [52–73%]	14/29 (48%) [29–67%]	24/35 (69%) [51–83%]	40/68 (59%) [46–71%]	11/13 (85%) [55–98%]	7/11 (64%) [31–89%]
Easily provoked laughter	55/91 (60%) [50–71%]	18/29 (62%) [42–79%]	22/34 (65%) [46–80%]	41/67 (61%) [49–73%]	8/13 (62%) [32–86%]	6/11 (55%) [23–83%]
Short attention span	84/91 (92%) [85–97%]	27/29 (93%) [77–99%]	33/35 (94%) [81–99%]	63/67 (94%) [85–98%]	12/13 (92%) [64–99.8%]	9/11 (82%) [48–98%]
Mid-face hypoplasia	17/92 (18%) [11–28%]	5/29 (17%) [6–36%]	7/35 (20%) [8–37%]	13/68 (19%) [11–30%]	2/13 (15%) [2–45%]	2/11 (18%) [2–52%]
Prognathism	19/92 (26%) [13–30%]	6/29 (21%) [8–40%]	7/35 (20%) [8–37%]	13/68 (19%) [11–30%]	3/13 (23%) [5–54%]	3/11 (27%) [6–61%]
Widely spaced teeth	57/90 (63%) [53–73%]	18/28 (64%) [44–81%]	21/35 (60%) [42–76%]	41/67 (61%) [49–73%]	9/13 (69%) [39–91%]	7/10 (70%) [35–93%]
Hair or skin color lighter than expected	29/91 (32%) [22–42%]	12/29 (41%) [24–61%]	12/34 (35%) [20–54%]	26/67 (39%) [27–52%]	3/13 (23%) [5–54%]	0/11 (0%) [0–28%]

UPD, uniparental disomy.

et al., 1994; Varela et al., 2004], 78% of those with UPD [Varela et al., 2004], and 100% of those with *UBE3A* mutations [Moncla et al., 1999a], although the number of participants in the two latter groups was relatively small. In contrast, fewer than 70% of our participants had easily provoked laughter. Of note, in a survey of 72 individuals between the ages of 5 and 33 years with AS due to a deletion, ''episodes of inappropriate laughter'' were reported in only 57% of them [Clarke and Marston, 2000], suggesting that such behavior may become less common with age [Pelc et al., 2008]. Although these individuals might laugh more readily than individuals without AS, some caregivers consider the laughter ''appropriate'' as long as a triggering stimulus can be identified. The true prevalence of ''frequent'' or ''inappropriate'' laughter recorded in surveys may therefore depend on the congruence between the investigators' and the parents' definitions of these terms. Moreover, it has been shown that children with AS initiate contact with adults, and smile at adults before being smiled at, more often than children with other intellectual disabilities of similar severity [Oliver et al., 2007]. This has led to the hypothesis that children with AS find interactions with adults unusually rewarding and use smiling and laughter to maintain their ''social resources,'' which further suggests that the laughter and smiles are not ''inappropriate'' [Oliver et al., 2007]. We have also observed that some of these children also tend to laugh when they are upset or fatigued, suggesting that laughter might be an ''emotional'' response to stress as well as a form of expression.

Fascination with water, another characteristic well known to the parents of children with AS, was observed in approximately 80% of our participants with deletions and 60–65% of those with either UPD/imprinting defects or *UBE3A* mutations, similar to reports from previous case series in which 68–79% of participants with deletions exhibited this trait [Clarke and Marston, 2000; Kara et al., 2008].

Mouthing of objects, although not often reported in case series, is said to occur in fewer than 80% of AS individuals [Williams et al., 2006]. However, it was reported in 95% of our participants, including 99% of those with a deletion. This difference may be due to the fact that the overall age of our subjects is younger than that of previous studies.

To assess the specificity of the various behavioral characteristics in AS, some authors have compared the prevalence of specific behavioral traits in individuals with AS to those with other intellectual disability syndromes. AS individuals were found to exhibit more mouthing behavior, hand-flapping, excitability, and had shorter attention span, and were more cheerful and less anxious, than those with Down syndrome and Prader–Willi syndrome [Walz and Benson, 2002]. Comparing AS individuals with deletions and UPD/imprinting defects to individuals with moderate or profound ''intellectual disability,'' Barry et al., found that significantly more AS individuals had mouthing behavior (76% vs. 43%) and sleep disturbances than the control group (68% vs. 26%), but significantly fewer AS individuals had short attention span compared to the control group (46% vs. 74%); there was no significant difference in the prevalence of ''laughs or giggles for no obvious reasons'' between AS individuals and the control group (49.2% vs. 49.6%) [Barry et al., 2005]. However, individuals with UPD/imprinting defects were over-represented (29%) in their cohort, and their control group was heterogeneous by design. A more recent study showed that individuals with AS had a stronger preference for, and attraction to, water-related activities compared to individuals with Down syndrome and those with ''non-specific intellectual disability'' [Didden et al., 2008].

We propose that the clinical suspicion for AS should be based on the neurobehavioral phenotype rather than specific dysmorphic features. The mid-face hypoplasia and prognathism depicted in standard textbooks were observed in fewer than 30% of our participants [Jones, 2006], and may be more characteristic of older individuals. Although behavioral attributes are often subjective and lack standardized operational definitions, identifying a ''behavioral phenotype'' can be useful for the diagnosis of some genetic syndromes such as Williams syndrome and Prader–Willi syndrome [Dykens, 1995; Finegan, 1998; Cassidy and Morris, 2002]. Our data, together with the findings of the comparative studies described above, suggest that the constellation of mouthing behavior, sleep difficulties, and fascination with water in a child with developmental delay, absent or minimal speech development, abnormal EEG, and an ataxic or broad-based gait should raise the suspicion for AS. More importantly, the absence of seizures or the lack of easily provoked or ''inappropriate'' laughter should not discourage consideration of this diagnosis. A few studies have suggested that the phenotypic features of AS change with age such that facial features become more prominent (though wide-spaced teeth become less common), and the attention span and sleep difficulties improve, but the fascination with water and EEG abnormalities persist [Buntinx et al., 1995; Laan et al., 1996, 1997; Smith, 2001]. In our cohort, participants with deletions were significantly younger than those in the non-deletion subclasses; however, other than having a higher prevalence of mouthing behaviors and lighter skin or hair color, and being less heavy and more microcephalic, there were no significant differences in the physical and behavioral traits between the deletion and the non-deletion subclasses.

The clinical phenotype of AS in the older individuals remains poorly defined, and it is hoped that as an increasing number of adults with AS enroll in natural history studies, we will learn more about these individuals and provide them with the most appropriate medical care. For example, although the majority of the participants in our cohort who were ambulatory had an ataxic or broad-based gait, we could assess this trait in only 41/92 (45%) of the participants because the rest had yet to develop the ability to walk. There is also a need to study greater numbers of individuals with the non-deletion subclasses to better understand the genotype–phenotype correlations in AS, as illustrated by the limited precision of our analyses.

In summary, we hope that this broad overview of the clinical features of AS will assist general pediatricians and pediatric subspecialists in identifying young children with AS, leading to earlier diagnoses and interventions. Having a definitive diagnosis could help these children obtain more intensive services through Early Intervention and other programs, thereby maximizing their developmental potential. It would also enable their parents and other family members to receive appropriate genetic counseling for future pregnancies. Healthcare providers should also be aware that children with AS due to UPD/imprinting defects are at risk of obesity and its complications.

ACKNOWLEDGMENTS

The authors are grateful to the General Clinical Research Centers (GCRC) at Children's Hospital Boston and Texas Children's Hospital for their support. They greatly appreciate: the study coordinators/nurses for their invaluable assistance—Beverly M. Feldman (Baylor College of Medicine), Vera Anastasoaie, Sharyn Lincoln, and Janette Z. Lawrence (Children's Hospital Boston), Fran Annese and Joy Graham (Greenwood Genetic Center), Marla Hashiguchi (Rady Children's Hospital San Diego); the clinical research staff (Jennifer Pilger, Rachel Richesson, and June T. Tran) at the Data Management Coordinating Center (DMCC) for their technical support; and the parents and guardians of the participants, along with the Angelman Syndrome Foundation, for their interest and devotion to this long-term study. We would like to thank Daniel Tarquinio for his help with computing growth centiles, and Alan K. Percy (University of Alabama at Birmingham) for his leadership and support. We are grateful to Donna Neuberg for her critical and helpful review of this manuscript. We would like to acknowledge the support of Mary Lou Oster-Granite (National Institute of Child Health and Human Development) for our work. WHT would like to thank Virginia E. Kimonis (now at University of California, Irvine) for her assistance in initiating the study at Children's Hospital Boston, and Alison Clapp, Medical Librarian at Children's Hospital Boston, for her assistance in obtaining the many references. The project described was supported by Grant Number NIH U54 RR019478 (awarded to A.L.B.), NIH U54 RR019259 (awarded to Jeffrey P. Krischer) from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), the NIH Office of Rare Diseases Research (ORDR), and the Angelman Syndrome Foundation—Western Area Chapter. Funding through NCRR was through the cooperative agreement mechanism. The protocol was reviewed and approved by the NCRR Protocol Review Committee and subsequent study progress was monitored by their Data Safety Monitoring Board (DSMB). Data were imported into the DMCC. Data analysis and interpretation was conducted cooperatively with the DMCC statistician (H-SL). Manuscript preparation, review, and approval were solely that of the authors. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of NCRR, ORDR, NIH, University of South Florida, or the Angelman Syndrome Foundation.

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