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CLINICAL REPORT

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Two Angelman families with unusually advanced neurodevelopment carry a start codon variant in the most highly expressed *UBE3A* isoform

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We present three children from two unrelated families with Angelman syndrome (AS) whose developmental skills are far more advanced than any other non-mosaic AS individual ever reported. All have normal gait and use syntactic language spontaneously to express their needs. All of them have a c.2T > C (p.Met1Thr) variant in *UBE3A*, which abrogates the start codon of isoform 1, but not of isoforms 2 and 3. This variant was maternally inherited in one set of siblings, but *de novo* in the other child from the unrelated family. This report underscores the importance of considering AS in the differential diagnosis even in the presence of syntactic speech.

KEYWORDS
child development, genetic association studies, inborn genetic diseases, rare diseases, siblings

1 | INTRODUCTION

Angelman syndrome (AS) is a neurodevelopmental disorder that results from a lack of expression of the maternally inherited *UBE3A* on chromosome 15q11–q13. This may be due to one of four molecular mechanisms, namely a deletion in the critical region of the maternally inherited chromosome 15 that encompasses *UBE3A*, paternal uniparental disomy, imprinting defects, and pathogenic variants in the maternally inherited *UBE3A*. The major characteristics of AS include global developmental delay, intellectual disability, ataxia, seizures, and very limited or a complete absence of speech (Tan et al., 2011). Through alternative splicing, *UBE3A* encodes 3 isoforms that differ in the length and sequence of the amino-terminus of the protein (Yamamoto, Huibregtse, & Howley, 1997). Isoform 1 encodes the shortest polypeptide, which is 850 residues in length. Isoforms 2 and 3 include these 850 residues, and an additional 23 and 20 residues, respectively, on the amino-terminus. Thus, each isoform has a distinct start codon (Figure 1a). Pathogenic variants in the start codon of either isoform 2 or 3 are predicted to only affect those specific isoforms, whereas a start codon pathogenic variant in isoform 1 would result in mutant proteins from all 3 isoforms. Although it remains unknown whether different isoforms have different biological functions *in vivo*, isoform 1 is the most highly

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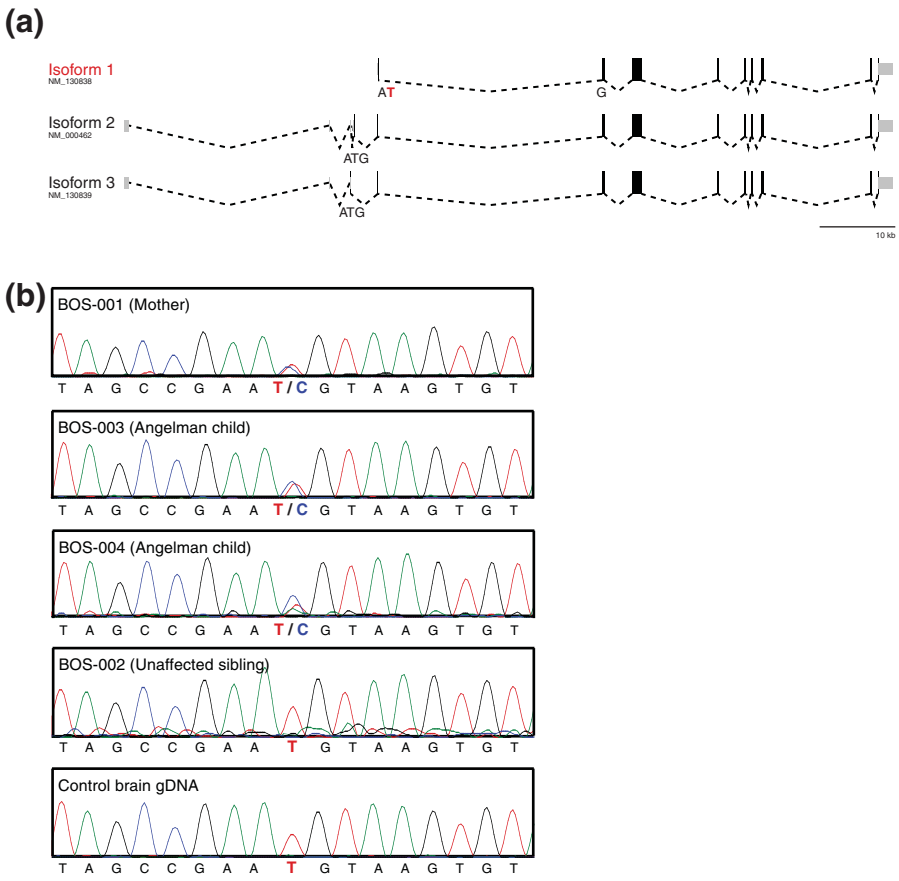

FIGURE 1 An isoform-specific *UBE3A* start codon variant in affected siblings. (a) The human *UBE3A* gene has 3 canonical transcript isoforms as indicted by RefSeq accessions. (b) Angelman siblings and their unaffected mother are heterozygous for a start codon variant in isoform 1 of *UBE3A* as shown by direct sequencing of this region of the genome. Each mixed peak was confirmed by sequencing of individual alleles; PCR amplicons (a mixture of maternal and paternal alleles) were cloned into a plasmid for colony sequencing of individual alleles. This start codon variant is not present in an unaffected sibling or an unrelated control. [Color figure can be viewed at wileyonlinelibrary.com]

expressed isoform across approximately 50 primary tissue types, including the cerebral cortex and cerebellum (Genotype-Tissue Expression database V6p, $n = 8555$ samples from 544 donors) (Mele et al., 2015).

Individuals with AS have severe motor and language delays. The highest level of both fine and gross motor skills that has been reported is 60 months (Beckung, Steffenburg, & Kyllerman, 2004). Language development is particularly impaired in AS individuals. Although receptive language is better developed than expressive language, the most advanced receptive language skill that has been reported is at the 24-month level (Andersen, Rasmussen, & Stromme, 2001). The highest level of expressive language that has been reported is at the 14-month level (Andersen et al., 2001), with only 18% of individuals with AS using spoken words to express their needs in an online survey of children and adults with AS, none of whom used more than 20 single words (Quinn & Rowland, 2017). There are no published cases of individuals with AS who are able to express themselves using two or more words in meaningful phrases or sentences, except in individuals with mosaic AS (Fairbrother et al., 2015; Le Fevre et al., 2017).

Through the AS Natural History study (ClinicalTrials.gov identifier: NCT00296764), we identified a pair of brother and sister with AS, referred herein as "Family 1," due to a *UBE3A* pathogenic variant who have motor and language skills that are more advanced than expected and who use spontaneous phrases to express themselves, which has never been reported in AS. By searching the ClinVar database (Landrum et al., 2018), we subsequently identified another child from an unrelated family, referred herein as "Family 2" (ClinVar Submission Accession: SCV000224679.3) who has the same *UBE3A* variant and a similar clinical phenotype.

2 | MATERIALS AND METHODS

2.1 | Neurodevelopmental assessments on the siblings (Family 1)

The history on the siblings in Family 1 was obtained by interviewing their mother using standardized questionnaires in the AS Natural History Study. The Vineland Adaptive Behavior Scales, second edition (Vineland-II) (Sparrow, Cicchetti, & Balla, 2005), a semi-structured interview that assesses a participant's adaptive skills across different developmental domains was administered by a psychologist (Sadhwani) to assess language and motor skills.

Additional standardized language testing was performed using the Preschool Language Scale, Fourth Edition (PLS-4) (Zimmerman, Steiner, & Pond, 2002), an in-person assessment of receptive and expressive language skills. Spontaneous language samples were also recorded through the evaluations. In addition, a speech and language pathologist with expertise in evaluating individuals with AS (Calculator) assessed these children and provided behavioral observations, clinical judgment, and inferences about their language level.

2.2 | Developmental history on the child from Family 2

The child identified through the ClinVar database had not had a formal standardized developmental assessment. The developmental milestones and behavioral characteristics of this child were obtained through a questionnaire that his mother completed.

2.3 | Amplicon and allele sequencing of *UBE3A* isoforms in Family 1

We extracted genomic DNA from buccal swabs on the siblings from Family 1. The region around the start codons of all *UBE3A* isoforms was amplified from the samples and from reference adult human brain genomic DNA (BioChain Institute, Inc., Newark, CA, USA) using Phusion Flash (Thermo Fisher Scientific, Waltham, MA, USA). Using two sets of primers (one to capture start codons of isoforms 2 and 3, and another for isoform 1), we sequenced the start codons of all 3 isoforms (Figure 1b) with the following primers:

Isoform 1 forward:
agtcacgacgttgtaaaacgacggccagtgCAACCTCCCTATTTCCCTACAACTGCTAC

Isoform 1 reverse:
caggaaacagctatgaccatgattacgccaGCTATCCAGTGCAAAACTTCACCTCAG

Isoform 2 and 3 forward:
agtcacgacgttgtaaaacgacggccagtgTGCCAAGTTGCTGGAAGTAAGAATCC

Isoform 2 and 3 reverse:
caggaaacagctatgaccatgattacgccaCCCTCCTTGGTGACTGATTGCTCTAT

In the primer design, regions annealing to the genome (in capital letters) were flanked with sequences for universal sequencing primers M13F and M13R (in lower case letters). To sequence individual alleles, we cloned PCR products into pUC19 and sequenced individual clones. Bacterial colonies were directly sequenced using rolling circle amplification followed by Sanger sequencing. Next-Generation Sequencing technology was not utilized in this study.

2.4 | Next-Generation Sequencing of selected genes, including *UBE3A*, in Family 2

A peripheral blood sample was obtained from the child in Family 2 and submitted to EGL Genetic Diagnostics (Tucker, GA) for clinical diagnostic testing. In solution hybridization and next-generation short base pair read sequencing of the coding exons of 63 genes associated with syndromic and non-syndromic forms of autism spectrum disorder, including *UBE3A* (EGL Genetics Autism Spectrum Disorders: Tier 2 Panel) was performed.

The AS Natural History study was approved by the Boston Children's Hospital Institutional Review Board, and informed consent was obtained from the mothers of these participants.

3 | RESULTS

3.1 | Clinical presentation: Family 1

3.1.1 | Sibling A

Sibling A was an 11-year-old boy who was born at 35 weeks' gestation with birth weight, length, and head circumference that were appropriate for gestational age. Clinical evaluation for global developmental delay led to the finding of a heterozygous pathogenic variant in *UBE3A*: c.2T > C (p.Met1Thr) [GenBank transcript: NM_130838.1, GRCh37 coordinate: chr15:25650608, dbSNP: rs587780577, ClinVar allele ID: 139903] that was inherited from his phenotypically normal mother, confirming the diagnosis of AS at the age of 36 months. This variant from this family was subsequently included in an article that reported *UBE3A* mutations that had been identified by various clinical laboratories (Sadikovic et al., 2014). He had never had any clinical seizures, but he had multiple nocturnal awakenings. His behavioral profile was characterized by having a happy disposition, easy excitability, easily provoked laughter and hand-flapping, hyperkinesia and having a short attention span; he did not have any fascination with water nor did he have any mouthing behavior. His facial appearance was consistent with that of AS with prominent cheekbones and prognathism that became more prominent with age.

Early developmental milestones were significantly delayed (Table 1). The results of developmental evaluations performed at the age of 11 years and 2 months are given in Table 2. His fine and gross motor skills were both at the equivalent of a 47 month-old. He had an essentially normal gait without any observable ataxia. His receptive language skills were at the equivalent of a 51 month-old on the PLS-4 and that of a 30 month-old on the Vineland-II, the discrepancy between which may be because the PLS-4 provides a more refined assessment of specific communication skills, while the Vineland-II assesses functional use of language in daily life.

Although he had access to a high-tech augmentative and alternative communication (AAC) device, he only used it in structured settings and rarely spontaneously. Speech was his primary method of communication, mainly with single words and 2–4 word phrases ("cut scissors," "I put on," "fly far far away," and "do it now") in a hypernasal voice, but with poor articulation. Behavioral inferences indicated that while his speech was more than 75% intelligible to familiar listeners when the context of the conversation was known, it was no more than 25% intelligible to unfamiliar listeners when the context was unknown. He typically required clarification from his caregivers in order to be understood.

TABLE 1 Developmental milestones (age at which a given skill was acquired) of sibling A and sibling B in Family 1 and child in Family 2

Developmental milestones	Family 1 Months		Family 2 Months
	Sibling A	Sibling B	
Gross motor			
Rolls back to front	7	9	[a]
Sit unsupported	13	7	[a]
Crawls on hands and knees	16	11	[a]
Pulls to stand	14	14	9
Walks with support	18	14	9
Walks independently	21	18	15
Walks upstairs	24	20	48
Walks downstairs	24	24	48
Pedals tricycle	48	48	48
Fine motor			
Holds small object	10	9	[a]
Reaches for object	12	8	[a]
Transfer hand-to-hand	14	10	[a]
Uses pincer grasp	10	12	Unknown[b]
Receptive language:			
Follows instruction when accompanied by gesture	30	18	[a]
Follows instruction without gesture	36	24	[a]
Expressive language			
Cooing/Sounds of pleasure	4	6	[a]
Gestures/Points to indicate want	21	24	[a]
Single words	36	24	12

[a]Child had these skills (by age 8½ years), but the age at which he acquired them was unknown.
[b]Unknown whether he was able to use a pincer grasp.

3.1.2 | Sibling B

Sibling B was the 9-year-old sister of sibling A. She was born at 38 weeks' gestation with birth weight and length that were appropriate for gestational age. Following the diagnosis of AS in her brother, she was tested at 23 months of age and found to have the same *UBE3A* variant. Like her brother, she had never had clinical seizures, but she had multiple nocturnal awakenings. Her behavioral profile was characterized by having a happy disposition, mouthing (but not eating) of non-food objects, and having a short attention span. However, she was not easily excitable and did not have easily provoked laughter, hand-flapping behavior, fascination with water, or hyperkinesia. Her facial appearance was also consistent with that of AS with prominent cheekbones and prognathism that became more prominent with age.

Her early developmental milestones were delayed but generally achieved at a slighter earlier age than sibling A (Table 1). The results of developmental evaluations at the age of 9 years and 10 months are given in Table 2. Fine motor skills were at the equivalent of a 66 month-old, while gross motor skills were at the 71 month-old level on the Vineland-II. Her gait was normal with no observable ataxia. Her receptive language skills were at the equivalent of a 63 month-old on the PLS-4 and a 34 month-old on the Vineland-II.

She also had access to the same AAC device, but speech was also her primary method of spontaneous communication, using sentences of up to 8 words to request and ask questions (e.g., "Hey guys, do you want to play catch?" "Who like baseball?" "You want to try," "Mommy I fix it," and "I want pink pencil and a spoon"). Her utterances were primarily telegraphic in nature, with certain sentence elements (e.g., verbs and modifiers) often deleted. Her speech sometimes included blended sounds with the ending of words clipped off. She was easier to understand than sibling A. It was estimated that an unfamiliar adult could understand 90% of her utterances when they knew the topic of the conversation and approximately 75% when they had no knowledge of the topic.

3.1.3 | Family history

The maternal grandparents were deceased and hence not available for testing. However, the mother's paternal aunt had a son with severe developmental delay especially in his expressive language, intellectual disability, a happy disposition, and he was easily excited, "always laughing," and exhibited hand-flapping behavior. He had never been tested for this *UBE3A* variant, but his mother and the mother of our siblings had long felt that his behavioral profile was similar to that of sibling A and could be consistent with AS.

3.2 | Clinical presentation: Family 2

The child in Family 2 was an 8½-year-old boy who was born at 36 weeks' gestation. His mother first noted that he was "weak" at the age of 6 months old. He subsequently had global developmental delays, particularly in expressive language. However, he was not diagnosed with AS until the age of 5 years and 4 months old, when he had a Next-Generation Sequencing panel test for autism spectrum disorders, and he was found to have the same *UBE3A* variant as that identified in Family 1.

Unlike the siblings in Family 1, he had had multiple types of seizures almost every day since he was 1½ years old despite treatment with clobazam and oxcarbazepine. His seizure control improved with vagal nerve stimulation, but he continued having absence seizures daily. He had multiple nocturnal awakenings, and on some nights, he also had prolonged sleep latency. His behavioral profile was characterized by being affectionate, being easily excitable, having an "extreme fascination" with water, and mouthing (but not eating) of non-food objects. He used to have unprovoked laughter for no apparent reason, but more recently, he would laugh only when he thought the circumstance was amusing. His facial characteristics were reminiscent of those seen in AS and of the siblings in Family 1, particularly with the prominent cheekbones, thin vermilion of the upper lip, and prognathism (Figure 2).

While his developmental milestones were delayed, he achieved some of his milestones at an earlier age than those seen in the siblings in Family 1 (Table 1). His gait was normal and he was able to walk up and down stairs alternating feet. He started using 2-word phrases at age 7, and by the age of 8½ years, he was speaking in short sentences with 3–4 words such as "I go home," "Change iPad dead," and "I want more juice." His mother estimated that he was using about 10 different

TABLE 2 Results of developmental evaluation (age equivalents) using Vineland adaptive behavior scales, 2nd edition (Vineland-II) and preschool language scale, 4th edition (PLS-4) on sibling A and B in Family 1

	Sibling A	Sibling B
Age at testing	11 years and 2 months	9 years and 10 months
Gender	Male	Female
Fine motor skills Vineland-II *(Example of skills achieved)*	47 months *(prints recognizable letters or numbers)*	66 months *(cuts simple shapes, ties a knot, uses a keyboard)*
Gross motor skills Vineland-II *(Example of skills achieved)*	47 months *(Walks up and down stairs alternating feet, catches a tennis ball)*	71 months *(Walks up and down the stairs alternating feet, hops and skips forward)*
Receptive language PLS-4 *(Example of skills achieved)*	51 months *(Understands body parts, prepositions, pronouns, quantity (e.g., more/most), shapes, time (e.g., day, night)*	63 months *(Understands body parts, colors, prepositions, pronouns, shapes, and time (e.g., day, night), quantity (e.g., more/most), seasons and sequence (e.g., first, last)*
VABS-II *(Example of skills achieved)*	30 months *(Follows two-step directions)*	34 months *(Follows two-step directions)*
Expressive language PLS-4 *(Example of skills achieved)*	27 months *(Labels objects, describes using single words, activities represented by images, answers the "what" and "where" questions)*	45 months *(Describes how an object is used, uses qualitative concepts (e.g., long, short), and answers "what" and "where" questions)*
VABS-II *(Example of skills achieved)*	34 months *(Identifies colors, uses prepositions, enunciate his first and last name upon request)*	48 months *(States the month and day of her birthday, modulates the tone, volume, and rhythm of her voice appropriately)*

phrases or sentences, and his speech was reportedly intelligible to unfamiliar listeners.

3.3 | *UBE3A* isoform analyses—Family 1

At the start codon of *UBE3A* isoform 1, both affected siblings and their mother were heterozygous for the T > C variant, whereas the unaffected sibling and the unrelated normal brain sample were homozygous for the reference allele (T). As a control, we also sequenced the start codon of isoforms 2 and 3, and we found that all samples matched the reference sequence (ATG).

Since the maternally inherited *UBE3A* variant disrupts the start codon of isoform 1, it may result in a complete absence of isoform 1 due to a lack of efficient translation initiation. In isoforms 2 and 3, the variant changes an internal methionine to threonine (p.Met24Thr and p.Met21Thr, respectively). The mutational impact of this variant was predicted to be deleterious for all 3 isoforms by the SIFT algorithm (Sim et al., 2012), the Ensembl Variant Effect Predictor (McLaren et al., 2016) and MutationTaster ($p > .99$; Schwarz, Rodelsperger, Schuelke, & Seelow, 2010). In agreement with this prediction, this variant is absent from the 1000Genomes, Exome Aggregation Consortium (ExAC), and Genome Aggregation (gnomAD) databases (1000 Genomes Project Consortium et al., 2012; Lek et al., 2016). Additionally, at the genomic level, the mutated codon is split between two exons with AT in one exon and the G in a downstream exon (Figure 1a). It is possible that the variant disrupts the splice site that is used in all isoforms. The observed phenotype in the affected siblings might be due to one or a combination of these consequences on the various *UBE3A* isoforms.

3.4 | Next-Generation Sequencing of selected genes—Family 2

The only reportable variant detected by clinical testing for a panel of genes associated with syndromic and non-syndromic autism in the child from Family 2 was the same *UBE3A* variant: c.2T > C (p.Met1Thr) [GenBank transcript: NM_130838.1]. Testing of both parents did not detect this variant, therefore, this variant was apparently *de novo* in this child. The possibility of germline mosaicism for this variant in either parent could not be excluded. No suitable polymorphic markers were identified in the genomic region around this variant to determine whether the variant was on the paternal or maternal allele. However,

FIGURE 2 Frontal view of child in Family 2 showing the facial features characteristic of Angelman syndrome, including prominent cheekbones, thin vermilion of the upper lip, and prognathism [Color figure can be viewed at wileyonlinelibrary.com]

based on the clinical phenotype of the child, we hypothesized that this variant was on the maternal allele since a child would not be expected to have an AS-like phenotype unless there is a pathogenic variant on the maternal *UBE3A* allele.

4 | DISCUSSION

We have identified a set of siblings and an individual from an unrelated family with AS whose motor and language skills are far superior to those in previously reported individuals with AS. Although the *UBE3A* variant in these three individuals has not been reported in other families with AS and there is no one clinical finding that is pathognomonic for AS, the constellation of intellectual disability affecting expressive language more than motor skills, multiple nocturnal awakenings, and behavioral characteristics such as easily provoked laughter, mouthing of non-food objects, and fascination with water is highly suggestive of AS, as are their facial features. To the best of our knowledge, these three individuals from two different families are the only non-mosaic AS individuals who can combine two or more words to generate phrases or sentences spontaneously, and they serve as a reminder that the presence of syntactic speech does not preclude a diagnosis of AS.

In considering the likelihood of this specific *UBE3A* variant being pathogenic and the sole etiology of the clinical phenotype in these three individuals, we note that the mode of inheritance in Family 1 would be consistent with that of AS, if the mother of those siblings indeed has a paternal first cousin with AS through her paternal aunt, acknowledging that this individual has not been tested for the presence of this variant. The finding of the same *UBE3A* variant in similarly affected individuals from two unrelated families further strengthens our hypothesis that this variant is indeed the cause of the clinical phenotype.

However, there is a phenotypic discordance between the siblings in Family 1 and their maternal first cousin once removed, with the latter being more severely affected; and there is also phenotypic discordance between the siblings in Family 1 and the individual in Family 2 in that the child in Family 2 has had refractory epilepsy since the age of $1\frac{1}{2}$ years old, whereas neither of the siblings in Family 1 have ever had seizures. Since none of the three affected individuals in this report have had whole exome/genome sequencing and we do not have functional data to provide support or otherwise for the pathogenicity of this *UBE3A* variant, we cannot disprove the alternative hypotheses for these observations, which include: (a) this *UBE3A* variant is benign and the true etiology in these individuals is due to a mutation in other gene(s); (b) the presence of a variant in a modifier gene that accounts for the difference in severity between the siblings in Family 1 and their maternal first cousin once removed; and (c) in the case of the child in Family 2, the presence of a second comorbid genetic disorder that results in intractable epilepsy, or the use of oxcarbazepine, which may exacerbate seizures in AS (Thibert, Larson, Hsieh, Raby, & Thiele, 2013; Valente et al., 2006). Recent studies have shown that about 4–7% of individuals with a genetic diagnosis identified through whole exome sequencing have a second comorbid genetic diagnosis that results in mixed or "blended" phenotypes (Balci et al., 2017; Posey et al., 2016; Yang et al., 2014), so it is certainly conceivable that the child in Family 2 might have an underlying genetic epilepsy disorder unrelated to AS.

The biological roles of each UBE3A isoform in the brain remain unknown. The *UBE3A* pathogenic variant in these individuals is predicted to abrogate the start codon of isoform 1, but the impact of this variant on isoforms 2 and 3 remains unclear. Further investigations into the expression of the different wild-type UBE3A isoforms in different brain regions and the level of expression of each isoform in the neurons of these three children, perhaps through the use of induced pluripotent stem cells, could potentially inform our understanding of the importance of the different UBE3A isoforms in the brain. Some of the therapeutic strategies that are currently being developed for AS involve reactivation of the normally silenced (i.e., imprinted) paternal *UBE3A* allele, but the level of *UBE3A* expression that would result in a clinically meaningful outcome is unknown. As such, knowing the minimum amount of UBE3A that needs to be expressed for an AS individual to have spontaneous syntactic speech would be important.

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CONFLICT OF INTEREST

None.

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