

Angelman syndrome: review of clinical and molecular aspects

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Abstract: “Angelman syndrome” (AS) is a neurodevelopmental disorder whose main features are intellectual disability, lack of speech, seizures, and a characteristic behavioral profile. The behavioral features of AS include a happy demeanor, easily provoked laughter, short attention span, hypermotoric behavior, mouthing of objects, sleep disturbance, and an affinity for water. Microcephaly and subtle dysmorphic features, as well as ataxia and other movement disturbances, are additional features seen in most affected individuals. AS is due to deficient expression of the ubiquitin protein ligase E3A (*UBE3A*) gene, which displays paternal imprinting. There are four molecular classes of AS, and some genotype–phenotype correlations have emerged. Much remains to be understood regarding how insufficiency of E6-AP, the protein product of *UBE3A*, results in the observed neurodevelopmental deficits. Studies of mouse models of AS have implicated *UBE3A* in experience-dependent synaptic remodeling.

Keywords: Angelman syndrome, chromosome 15q11-13, *UBE3A*, imprinting

Introduction

Harry Angelman, an English pediatrician, first described this condition in 1965 when he reported three children that he referred to as “Puppet Children” because of their unusual arm position and jerky movements.¹ In addition to the characteristic movements, Angelman noted severe intellectual disability, absent speech, and bouts of inappropriate laughter. In the nearly 50 years since that original report, the AS phenotype has been elaborated, and the etiology of the disorder identified as deficiency of *UBE3A*.^{2,3} The molecular pathogenesis of how *UBE3A* deficiency leads to this phenotype is beginning to be clarified. What follows is a description of our current understanding of the clinical and molecular aspects of AS.

Clinical review

“AS” is a neurodevelopmental disorder whose main features are intellectual disability, lack of speech, seizures, and a characteristic behavioral profile.^{4–9} It has a prevalence of between 1/10,000 and 1/20,000 individuals.^{10,11} See Table 1 for consistent, frequent, and occasional features of AS.

Performance

Development delays in AS are usually evident within the first year of life, with delayed attainment of gross motor, fine motor, receptive language, expressive language, and social skills. Reportedly, individuals with AS plateau at a developmental level of between 24 and 30 months,¹² and cognitive performance is usually in the range of severe functional

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Table 1 Clinical features of Angelman syndrome

Consistent	Frequent	Occasional
Functionally severe intellectual disability	Microcephaly with flat occiput/occipital groove	Scoliosis
Movement/balance disorder	Seizures	Hypopigmentation
Speech impairment	Abnormal EEG	Increased sensitivity to heat
Behavioral phenotype (easily excited, happy, frequent laughter, hypermotoric)	Gastrointestinal difficulties (feeding problems, gastroesophageal reflux, constipation)	Growth disturbance depending on genotype
	Fascination with water or crinkly items	Ocular problems (refractive and alignment errors)
	Mouthing behavior	
	Ankle pronation Sleep disturbance	

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Abbreviation: EEG, electroencephalogram.

impairment.¹³ Language development in those with AS is significantly impaired. Most individuals lack speech entirely, a few individuals have small single-word vocabularies,¹³ and rare individuals are able to use phrases. The combination of deficits exhibited by individuals with AS make the commonly used developmental assessment tools difficult to apply, and these tests underestimate the abilities of AS children (author's personal observations). Receptive language is superior to expressive language.¹⁴ Nonverbal communication using a variety of systems (picture exchange cards, communication devices, modified sign language) is possible in a substantial proportion of individuals with AS (author's personal observations). Although all skills are delayed, there is variability in adaptive behavior functioning, with relative strength in socialization and relative weakness in motor skills.^{12,14} A substantial subset of children with AS qualify for a comorbid diagnosis of autism,^{15–21} independent of the severity of their cognitive and adaptive behavior functioning.²¹ AS adults are not able to live independently, but most are able to use feeding utensils and perform some household tasks with supervision.^{22–25} Dressing skills are dependent on the degree of fine-motor dexterity. Daytime continence is possible with prompted voiding and habit training.

Seizures

Seizures occur in 80%–95% of children with AS and usually start in childhood.^{26–29} The onset of seizures is before age 3 years in 75% of affected individuals.²⁹ Seizure types include myoclonic, atypical absence, generalized

tonic–clonic, and atonic (“drop”) seizures. Many individuals exhibit multiple seizure types.^{27,28} Seizures usually require broad-spectrum anticonvulsant medication and often combination therapy. Efficacy appears to be highest with valproate and clonazepam and lowest with phenobarbital and carbamazepine;²⁸ vigabatrin and carbamazepine may exacerbate seizures.^{26,29} Some patients have responded to vagal nerve stimulation or ketogenic diet³⁰ for seizures that were medically refractory.^{28,29} There can be attenuation of seizure activity in adolescents.^{27–29} There is a characteristic electroencephalogram (EEG) “signature” in AS,^{31,32} which can sometimes be useful in pointing toward the diagnosis.³¹ Various combinations of very high amplitude rhythmic (primarily anterior) delta activity, diffuse high amplitude rhythmic theta activity, and posterior-predominant spike and sharp waves are seen in >90% of individuals with AS.^{33–38}

Behavior

The behavioral features of AS include a happy demeanor, easily provoked laughter, short attention span, hypermotoric behavior, mouthing of objects, sleep disturbance with reduced need for sleep, and an affinity for water.^{39–41} Though infancy can be difficult due to feeding problems and general irritability, happy disposition and increased smiling characterize most children.³⁹ Rarely, unhappy or irritable affect persists, and gastrointestinal difficulties such as dysmotility and gastroesophageal reflux disease may play a role (author's personal observations). Mouthing of objects becomes very prominent in the young child, along with drooling and tongue thrusting; these behaviors can be lessened or extinguished with behavioral modification. Individuals with AS have an apparently increased desire for social interaction.³⁹ Children are described as easily excited. Though paroxysms of laughter are said to occur in AS, the laughter is not truly “unprovoked”, since an inciting event can usually be identified; however, the responding laughter is frequently excessive or inappropriate to the triggering stimulus. The majority of AS patients exhibit a short attention span, though this characteristic does not discriminate from other conditions with intellectual disability,⁴² and most children are hypermotoric/hyperactive, becoming calmer in adolescence and adulthood. Disruptive behaviors^{43,44} are displayed by the majority of patients, including biting, pinching, hair-pulling, and grabbing. Rarely are these behaviors intended to cause harm; they usually result from easy excitability, desire for attention, poor control over movements, reduced repertoire of need expression, and occasionally frustration over an inability to communicate effectively. Behavioral noncompliance,

tantrums, and repetitive or stereotyped behaviors have also been described.^{43,44}

Sleep

Most children have an apparently reduced need for sleep (sometimes as little as 5–6 hours per night) and abnormalities of the sleep–wake cycle, with long or frequent periods of wakefulness during the night.^{45–47} Sleep problems can involve the initiation and/or maintenance of sleep and early morning awakening.^{45–48} In a small study, melatonin levels were found to be low in those with AS,⁴⁹ corroborating prior observations that melatonin improves the sleep of children with AS.^{50,51} Despite sleep disruption, most individuals with AS do not exhibit daytime somnolence. With behavior modification⁵² and/or pharmacologic treatment, sleep difficulties can be overcome in most patients, and sleep patterns improve with age.⁴⁸ Epilepsy severity correlates with sleep problems, but whether more severe seizures create sleep disturbance or whether poor sleep patterns exacerbate epilepsy remains unclear.⁵³

Other

Growth in AS varies with molecular diagnosis (see the “Genotype–phenotype correlations” section), and microcephaly is common (80%).⁵⁴ Individuals with AS are generally non-dysmorphic as infants, but a subtle craniofacial phenotype develops with time, consisting of midface recession, prognathism, and broad mouth (the latter two are possibly consequences of tongue thrusting, mouthing behaviors and increased smiling). A subset of patients have hypopigmentation of the skin, hair, and eyes; this is more common in those with a deletion,⁵⁵ who lack the maternal copy of *OCA2* and presumably have a hypomorphic allele on the paternal chromosome.⁵⁶ Patients with AS and oculocutaneous albinism type 2 (*OCA2*) have been reported, mostly commonly due to deletion of the maternal *OCA2* and mutation in the paternal *OCA2*.⁵⁷ A non-deletion patient with AS and *OCA2* presumably has this constellation due to mutation in the paternal *OCA2* and isodisomy for paternal chromosome 15 (author’s personal observations). AS patients with *UBE3A* mutations may have hypopigmentation on the basis of *UBE3A*’s regulation of the melanocortin-1 receptor, which is downregulated in *Ube3a* null mice.⁵⁸

Ocular problems in AS include refractive errors (usually hyperopia and astigmatism), iris and choroidal hypopigmentation, and esotropia or exotropia.^{59–61} Nystagmus is reported but is not common.⁶¹ Ocular hypopigmentation is seen in all molecular classes but is more common in those with

deletion.⁶¹ Patchy retinochoroidal atrophy was reported in two adults with AS.⁶² The author knows of one deletion patient with bilateral ocular pterygia requiring corneal transplantation to restore vision.

Truncal hypotonia and distal extremity hypertonia/hyperreflexia characterize the neurological examination of children with AS.⁶³ Movement disturbances, abnormalities of tone, and impaired balance contribute to the delayed acquisition of motor skills (sitting after 12 months, walking between 2 and 6 years). Movement disorders include jerkiness, ataxic gait, and tremors.^{63,64} Many walk with arms held up and flexed at the elbows, true to the original description. The incidence of nonambulation is said to be 10%,⁶⁵ but whether this will remain true in the modern era (of earlier diagnosis and prompt intervention and continued therapy) remains to be seen. The early institution and continuation of physical therapy may change the natural history of scoliosis, previously reported to occur in 10% of children and up to 70% of adults,⁶⁶ by improving truncal tone.

Life expectancy appears to be normal;^{22–26} however, early death by accidental drowning has claimed the lives of some children, and the author is aware of premature deaths due to choking, pneumonia, suffocation, and seizures (personal communication).

Genetic basis

AS is caused by a lack of expression of the maternally inherited *UBE3A* gene in the brain. *UBE3A* is one of a small subset of human genes that are imprinted – that is, expressed depending on parent of origin, in a tissue-specific manner.^{65,66} While in most tissues, *UBE3A* appears to be expressed from both alleles (though perhaps unequally favoring the maternal allele⁶⁷), in the brain, the paternally derived *UBE3A* gene is silenced, and only the maternally inherited copy is active.^{68–70} *UBE3A* was initially discarded as a potential candidate for the AS gene because it appeared not to be imprinted when studied in lymphocytes and fibroblasts,⁷¹ and its widespread expression ran counter to expectation since the phenotype is exclusively neurological. Establishing brain-only imprinting of *UBE3A* in mice resurrected *UBE3A*’s status as a candidate gene for AS.⁷⁰ Analysis of *Ube3a* in AS patients with biparental contribution to 15q11-q13 and no imprinting abnormalities showed point mutations in several unrelated patients, identifying it as the gene responsible for the AS phenotype.^{2,3}

AS is caused by deficient expression of the maternal copy of the *UBE3A* gene due to one of four molecular etiologies: deletion of the AS critical region on maternal

chromosome 15q11-q13, paternal uniparental disomy (UPD) for chromosome 15, an imprinting defect causing lack of expression of the maternal copy of *UBE3A*, and mutations in the maternally inherited copy of *UBE3A*.⁵⁵ There is a subgroup of patients with a clinical diagnosis of AS for whom no abnormality of *UBE3A* can be identified. Two potential explanations for these test-negative patients are 1) novel mechanisms for repression of *UBE3A* expression yet to be identified and 2) misdiagnoses of phenotypically similar conditions. Patients with AS and negative molecular analyses are now being recognized to have a variety of Angelman-like syndromes.^{72,73}

In a cohort of AS patients participating in a natural history study conducted as part of the Rare Diseases Clinical Research Network, the distribution of molecular diagnoses among 286 patients was as follows: 31.1% class II deletion, 23.8% class I deletion, 10.8% unspecified deletion, 3.8% atypical deletion, 8.7% UPD, 7.7% imprinting defect, and 11.2% *UBE3A* mutation (unpublished data). Among individuals with a deletion, 40.5% have a common 5.9 megabases (Mb) (class I) deletion; 53% have a smaller 5.0 Mb (class II) deletion, differing only by the location of the proximal (centromeric) breakpoint; and 6.5% have atypical deletions. Deletions are mediated by homologous misalignment and meiotic recombination between low-copy-number repeats (duplicons) that have been identified in proximal and distal 15q11-q13. These duplicons arose with the amplification of an ancestral gene, homologous to the E6-AP carboxy terminus (HECT) and regulator of chromatin condensation 1-like domain (RLD)-containing E3 ubiquitin protein ligase 2 (*HERC2*). Duplicons having 90%–99% identity to the first 79 exons of *HERC2* are found in at least ten copies in the 15q11-q13.⁷⁴

Paternal UPD is isodisomic in almost all cases. The most likely origin of this event is maternal nondisjunction producing a monosomy 15 conception, with post-zygotic rescue by duplication of the paternal chromosome 15.⁷⁵ As in the deletion cases, this class of AS represents de novo mutational events and has a very low risk for recurrence.

“Imprinting defect” occurs when a paternal imprint is erroneously assigned to the maternally inherited allele. Two types of imprinting defects are known: those due to a submicroscopic deletion of the imprinting center, and those with no detectable mutation.^{76–78} Most submicroscopic imprinting center deletions are familial and carry a 50% risk for recurrence. Thus far, all imprinting defects with undetectable mutations have been sporadic events. They are presumed to result from failure to establish or maintain the imprint dur-

ing oogenesis, due to a stochastic event or perhaps (as yet unknown) environmental factors.⁷⁸

Intragenic *UBE3A* mutations include insertion, deletion, nonsense, missense, and splice site mutations.^{79,80} A substantial portion of *UBE3A* mutations are inherited from the mother’s paternally acquired allele. In this circumstance, a 50% recurrence risk pertains.⁸¹

Mechanisms of imprinting and gene regulation

Genetic imprinting is the process of conferring functional differences onto specific genes such that their expression occurs from only one parent’s allele.⁶⁵ There is incomplete understanding of the mechanism(s) of imprint establishment in the germ line, imprint maintenance during development and postnatal life, and imprint reversal in the germ line of the next generation. Further complexities, such as tissue-specific imprinting and age-related changes in imprinting are poorly understood.

Mechanisms of controlling gene expression include DNA insulators (DNA elements which prevent nearby chromatin domains from interacting); histone modifications (such as acetylation, phosphorylation, and methylation), which alter chromatin structure and influence transcriptional accessibility; DNA methylation; and transcriptional enhancer competition (promoters of linked imprinted genes competing for access to enhancers).^{82–84} Each of these epigenetic mechanisms overlays the information contained within the nucleotide sequence. Imprinted genes are found in clusters in specific areas of the genome, suggesting coordinated regulation by a regional element, which has been designated the “imprinting control region” (ICR).

DNA methylation is the most well understood of the recognized epigenetic mechanisms. The addition of a methyl group to the cytosine base of a CpG dinucleotide is found in most imprinted genes and in all ICRs.⁸² Loss and reacquisition of DNA methylation occurs during specific phases of germ cell development, and probably represents erasure of the imprint from the previous generation and re-establishment of the parent-of-origin specific epigenotype. DNA methylation may play a role in establishing and/or maintaining the imprint.^{82–84}

Deficits in the imprinted gene cluster on chromosome 15q11-13 cause Prader–Willi syndrome (PWS) and AS.^{7,66,78,85,86} Loss of expression of maternally derived *UBE3A* causes AS, while loss of expression of paternally derived gene(s) causes PWS. The main features of PWS are neonatal hypotonia and failure to thrive, childhood onset hyperphagia and obesity, small hands and feet, short stature,

hypogonadism, and cognitive impairment.⁸⁷ Though many features of PWS can be reproduced by absence of a small nucleolar organizing RNA gene, *SNORD116*, the full PWS phenotype requires the loss of expression of several genes, indicating that PWS is truly a contiguous gene syndrome.⁸⁷

Differential methylation of chromosome 15q11-q13 provides the basis for diagnostic testing for PWS and AS. The maternal chromosome is highly methylated while the paternal chromosome is mostly unmethylated in the 15q11-q13 region, and this can be demonstrated using methylation-sensitive restriction enzymes and Southern blot analysis or polymerase chain reaction assay of the promoter region of the small nuclear ribonucleoprotein polypeptide N (*SNRPN*) gene. A maternal-only contribution is diagnostic of PWS, while an exclusively paternal contribution indicates AS. A normal methylation profile having both a maternal and paternal contribution excludes PWS but does not rule out AS, because 10%–20% of cases of AS are due to maternally inherited *UBE3A* mutations.^{79,80}

The ICR for the 15q11-q13 cluster has been designated the “IC”.^{76,85} It was defined by small submicroscopic deletions in a subgroup of PWS and AS multiplex families that demonstrated biparental inheritance of the 15q11-13 region yet uniparental imprint.⁷⁶ The IC regulates in cis the establishment and maintenance of the imprint for the entire cluster. The IC has a bipartite structure, the Prader–Willi syndrome imprinting center (PWS-IC) and the Angelman syndrome imprinting center (AS-IC), separated by 35 kb (Figure 1). All AS patients with IC deletions are missing the AS-IC (880 bp centromeric of PWS-IC).⁸⁸ The PWS-IC establishes and maintains paternal gene expression. During oogenesis, the AS-IC negatively regulates the PWS-IC and prevents the paternal imprint from being established. Deletion of the AS-IC leads to a paternal imprint of the entire 15q11-q13 region, and AS occurs when this is transmitted through the maternal germ line. The PWS-IC and AS-IC must be proximal and correctly related in order for the maternal imprint to be established.⁸⁹

The genes makorin ring finger protein 3 (*MKRN3*), necdin (*NDN*), melanoma-associated antigen-like 2 (*MAGEL2*) and *SNRPN* upstream reading frame (*SNURF-SNRPN*) are expressed only from the paternal chromosome where their promoter regions are unmethylated.^{66,90,91} Between *SNURF-SNRPN* and *UBE3A* are located more than 70 small nucleolar RNA (snoRNA) genes. These noncoding genes produce snoRNAs, which modify ribosomal RNA.⁶⁶ Though not differentially methylated, snoRNAs in this region are indirectly under the control of methylation, because they are processed

from the differentially methylated and paternally expressed *SNURF-SNRPN* sense/*UBE3A* antisense transcript.

Studies of the orthologous mouse region (chromosome 7) form the basis for much of what is known about methylation status and imprinting of human chromosome 15q11-q13. However, important differences exist.⁹² The *Frat3* gene, for which there is no human homologue, has joined the mouse PWS/AS region, acquiring the paternal pattern of methylation and expression. Transgenic studies, where human elements of the PWS/AS region are inserted in the mouse, have shown that the regulatory elements of the imprinting machinery have diverged between the two species. No mouse equivalent of the human AS-IC has been identified. Further study of the mouse PWS/AS region imprinting will probably yield important insights,⁹³ but some may not apply to human *UBE3A* imprinting.

UBE3A itself is not differentially methylated;⁵⁵ its imprinted expression is indirectly regulated by a long non-coding antisense RNA transcript (*UBE3A-ATS*) which is part of a larger *SNURF-SNRPN* transcript.⁶⁶ *UBE3A-ATS* is active on the paternal chromosome and blocks *UBE3A* transcription in cis.^{94–97} Changes in DNA methylation and histone acetylation of the PWS-IC control production of the *UBE3A-ATS* from the paternal allele.⁹⁸ The mechanism by which *UBE3A-ATS* blocks *UBE3A* transcription is unknown, but may involve histone-mediated repression, transcriptional interference, or repressive three-dimensional chromatin structure.⁶⁶

Molecular pathogenesis

UBE3A spans 120 kb of genomic DNA.⁹ Mutations have been detected throughout all regions of the gene. Sixteen exons have been identified; since this region displays alternative splicing, additional exons at the 5′ end of the gene are possible. *UBE3A* is transcribed in the direction from telomere to centromere, producing RNA transcripts of 5–6 kb that include 2 kb of 3′ untranslated region sequence. There are three main transcripts producing three isoforms of *UBE3A*, and eight to ten additional transcripts of uncertain function. The function of the three different isoforms of *UBE3A* is unknown and the significance of tissue-specific variations in RNA splicing and isoform predominance is unclear.

UBE3A encodes E6-associated protein (E6-AP), an E3 ubiquitin ligase.⁹ E6-AP derives its name from its initial characterization, in which it was found to be associated with the E6 protein of papillomaviruses to promote degradation of p53. However, E6-AP does not maintain a stable association with p53, and its main function is believed to be participation in protein degradation in proteasomes via the ubiquitin pathway. The ubiquitin-proteasome system targets cellular proteins for

destruction by covalently attaching ubiquitin to one or more lysine residues of proteins destined for degradation. Ubiquitination involves a three-step process: 1) activation of ubiquitin by an E1 enzyme, 2) transfer to an E2 conjugating enzyme, and 3) covalent ligation of ubiquitin to the protein substrate by an E3 ligase; E6-AP is one of many E3 ligases. E6-AP also interacts with proteins involved in such cellular functions as cell-cycle regulation and synaptic function and plasticity,^{99,100} and acts as a transcriptional co-activator of steroid hormone receptors.^{101,102} The C-terminus of E6-AP is a functionally important and highly conserved domain that is shared by a family of proteins (HECT domain), of which E6-AP is the founding member. The last six amino acids of the E6-AP C-terminus are essential for activity in vitro. It is unknown how substrate specificity is determined. E6-AP is found in all tissues that have been studied.

Presumably, defective UBE3A activity results in failure to degrade its substrates because of impaired ubiquitination. Several E6-AP targets have been identified, including activity-regulated cytoskeleton-associated protein (Arc) and Ephexin5.⁹ E6-AP regulates Arc levels either by regulating estradiol-induced transcription of Arc¹⁰³ or by direct ubiquitination of Arc.¹⁰⁴ Arc regulates surface expression of alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionate receptors (AMPA receptors). When E6-AP is deficient, the excitatory postsynaptic AMPARs are internalized,

which impairs synaptic transmission.¹⁰⁴ Ephexin5 has a role in controlling synapse number.¹⁰⁵ Recently proposed as a substrate for E6-AP is saccin,¹⁰⁴ defects of which cause a form of spastic ataxia;¹⁰⁶ given the ataxic gait in AS, saccin is an attractive target. There is also mounting evidence that UBE3A and methyl CpG binding protein 2 interact to regulate the expression of target genes.¹⁰⁷ Recently, a role for E6-AP in Golgi acidification and protein sialylation was proposed.¹⁰⁸

Mouse models have facilitated the progress in understanding the molecular pathogenesis of AS.¹⁰⁹ The *Ube3a* knockout mouse, generated with target disruption of *Ube3a* on the maternal chromosome 7, nicely recapitulates the human disorder.^{110,111} *Ube3a*^{mat-pat} mice demonstrate reduced brain size, ataxia, motor impairment, abnormal EEG, sleep disturbance,¹¹² learning and memory impairment, and deficits in hippocampal long-term potentiation (LTP).^{110,111} In the hippocampus of *Ube3a*^{mat-pat} mice, the calcium/calmodulin-dependent protein kinase II (CaMKII), which plays a role in induction of LTP that is critical for memory, has an increased level of inhibitory phosphorylation and reduced activity.¹¹³ *Ube3a*^{mat-pat} mice with a concomitant mutation in CaMKII that blocks inhibitory phosphorylation are indistinguishable from wild-type mice,¹¹⁴ indicating the defect of E6-AP can be overcome with excess CaMKII activity. E6-AP appears to play a role in activity-dependent synaptic

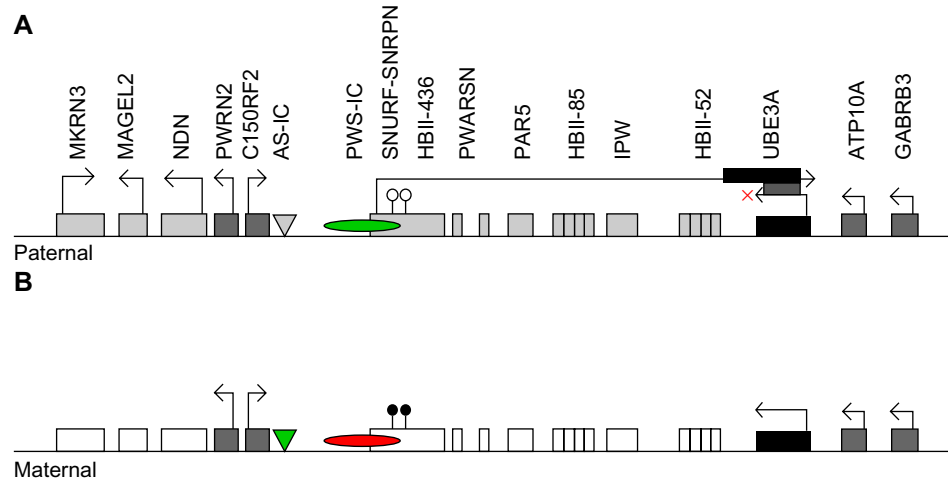


Figure 1 Imprinting map of the human chromosome 15q11-13 region. Paternal and maternal chromosome 15q11-13 regions around the Angelman syndrome imprinting center (AS-IC) and Prader–Willi syndrome imprinting center (PWS-IC) are represented in (A and B), respectively.

Notes: Paternally expressed genes (gray boxes), maternally expressed genes (black boxes), maternally repressed genes (white boxes), and biallelically expressed genes (dark gray boxes) are represented with arrows marking transcription start sites. Right arrow indicates gene transcription on “+” strand, whereas left arrow indicates gene transcription on the “-” strand. AS-IC (triangle) and PWS-IC (ellipse) are shaded depending on histone modification in the area. AS-IC is dormant (gray triangle) on the paternal chromosome, whereas, on the maternal chromosome, it is acetylated and methylated at H3-lys4 (green triangle), thus active. PWS-IC is active on the paternal chromosome (green ellipse), since it is also acetylated and methylated at H3-lys4. However, PWS-IC at the maternal chromosome is methylated at H3-lys9 and repressed (red ellipse). Differentially, the CpG methylated region (differentially methylated region 1 [DMR1]) in small nuclear ribonucleoprotein polypeptide N (SNRPN) exon 1 partially overlaps with PWS-IC. Note that DMR1 on the maternal but not paternal chromosome is methylated (black pin). Ubiquitin protein ligase E3A antisense transcript (*UBE3A-ATS*) originating upstream of SNRPN can either be a degradable complex with *UBE3A* transcript or prevent the extension of the ubiquitin protein ligase E3A (*UBE3A*) transcript (collision or upstream histone modifications represented by “X”). Reproduced from Jana NR. Understanding the pathogenesis of Angelman syndrome through animal models. *Neural Plast.* 2012;2012:710943.¹³⁷

Abbreviations: MKRN3, makorin ring finger protein 3; NDN, necdin; MAGEL2, melanoma-associated antigen-like 2; SNURF-SNRPN, small nuclear ribonucleoprotein polypeptide N upstream reading frame; PWS-IC, Prader–Willi syndrome imprinting center; UBE3A, ubiquitin protein ligase E3A; PWRN2, Prader–Willi Region Non-Protein Coding RNA 2; HBII-436, Small Nucleolar RNA, C/D Box 107; PAR5, Prader Willi/Angelman Region RNA 5; IPW, Imprinted In Prader–Willi Syndrome (Non-Protein Coding); HBII-52; Small Nucleolar RNA, C/D Box 115; ATP10A, ATPase, Class V, Type 10A; GABRB3, Gamma-Aminobutyric Acid (GABA) A Receptor, Beta 3; PWARSN, Prader Willi/Angelman region RNA, SNRPN neighbor; C15ORF2, Chromosome 15 Open Reading Frame 2; HBII-85, cluster of C/D box containing small nucleolar RNAs.

plasticity; that is, the remodeling of synapses depending on experience.^{99,100} The observations of abnormal dendritic spine morphology¹¹⁵ and increase in synapses lacking AMPARs¹⁰⁴ in *Ube3a*^{m-p+} mice fit with this hypothesis. *Ube3a* acts as a transcriptional co-activator of many steroid hormone receptors, including the glucocorticoid receptor, and glucocorticoid receptor-mediated signaling is dysregulated in the brains of *Ube3a*^{m-p+} mice, which show increased serum levels of corticosterone and increased anxiety-like behavior.¹¹⁶ *Ube3a*^{m-p+} mice also have enhanced neuregulin-ErbB4 signaling¹¹⁷ that correlates with abnormal synaptic plasticity and memory impairment, not mediated through differences in AMPARs and N-methyl-D-aspartate receptors and not mediated through direct interaction of neuregulin or ErbB4 with UBE3A. The deficits in LTP can be rescued by ErbB inhibitors infused directly into the hippocampus of *Ube3a*^{m-p+} mice.¹¹⁷

Diagnostic algorithm

The most sensitive test for AS is a methylation analysis of the chromosome 15q11-13 region, using either methylation-specific polymerase chain reaction or methylation-sensitive multiplex ligation-dependent probe amplification. Further testing is needed to parse those with abnormal methylation testing into deletion, UPD, and imprinting defect categories.¹¹⁸ Figure 2 depicts the diagnostic algorithm for testing for AS.

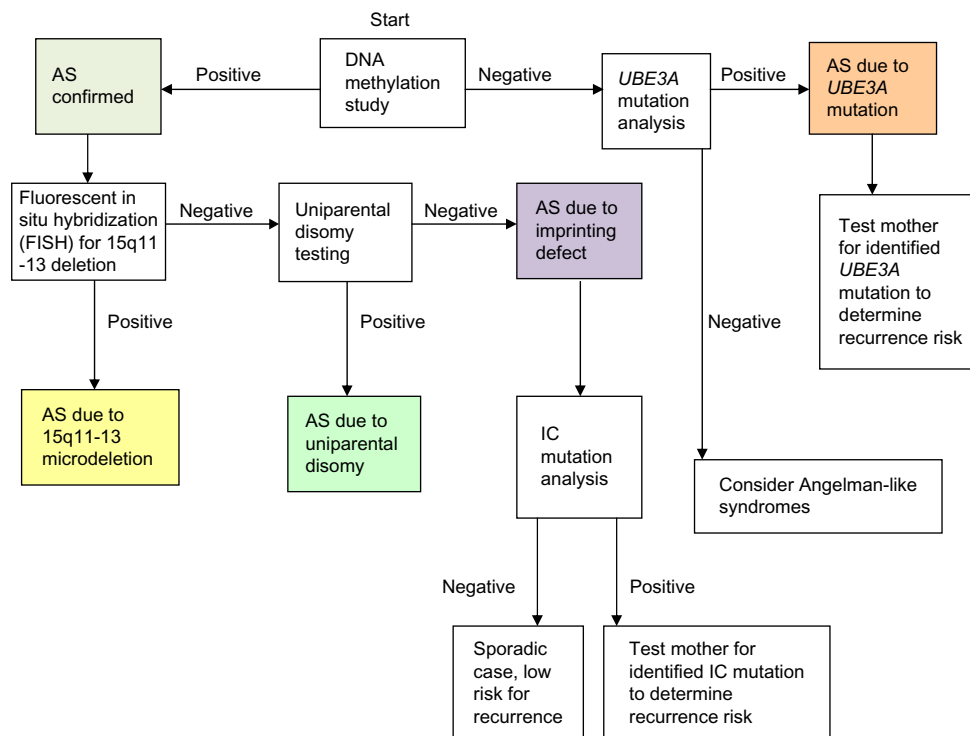


Figure 2 Molecular diagnostic algorithm for AS.

Abbreviations: AS, Angelman syndrome; IC, imprinting center.

If DNA methylation analysis is negative, then *UBE3A* sequencing is appropriate for those with a convincing AS phenotype. When both DNA methylation analysis and *UBE3A* mutation testing are negative, the likelihood of AS is small.¹⁸ Though once thought to represent a substantial portion of cases, test-negative (“clinical diagnosis”) patients with AS are probably rare. Patients with an AS phenotype in whom testing returns normal should be considered for an alternative diagnosis, such as Pitt–Hopkins syndrome, Mowat–Wilson syndrome, Kleefstra syndrome, Phelan–McDermid syndrome, Koolen–de Vries syndrome, Christianson syndrome, and *MBD5* haploinsufficiency.^{72,73}

Recurrence risk for AS due to microdeletion or UPD is negligible, whereas *UBE3A* mutations and imprinting defects can have a 50% risk for recurrence if the mother is found to carry the mutation on her paternally inherited chromosome 15.⁸¹

Genotype–phenotype correlations

Clinical differences between the molecular classes of AS have been recognized.^{55,119–123} Those with deletion tend to be shorter and lighter than the general population, while those with UPD or imprinting defects tend to be taller and heavier; growth in those with *UBE3A* mutations is variable.⁵⁴ There is a higher incidence of microcephaly in the

deletion group compared with non-deletion subtypes.^{54,119} As a group, patients with deletions tend to have a more severe phenotype (later onset of independent walking, earlier onset and increased severity of seizures, complete absence of speech) than patients with UPD, imprinting defect, or *UBE3A* mutation (non-deletion patients).^{14,55} In younger patients with AS, those with *UBE3A* mutations scored higher in tests of cognition, gross motor and fine motor skills, and receptive language than deletion patients.¹⁴ Though speech is usually absent in AS patients with deletions, use of up to 20 words has been reported in AS patients of other molecular classes; however, Gentile et al found no differences in expressive language skills in patients of less than 5 years of age with AS with respect to molecular subtype.¹⁴ Compared with patients with a smaller class II deletion (~5 Mb), patients with a larger class I deletion (~6.0 Mb) are more likely to meet criteria for a comorbid diagnosis of autism, have lower cognitive scores, and require more seizure medications.^{122–124} It has been proposed that other deleted genes in the 15q11-13 region account for the increased phenotypic severity, but the precise contribution and mechanisms remain to be clarified. Three genes for gamma-Aminobutyric acid (GABA)-receptor subunits (*GABRB3*, *GABRA5*, *GABRG3*) are located telomeric of *UBE3A* and contained within the deleted region. Though these genes show biallelic expression, a role for them in the genesis of epilepsy in AS has been suggested.¹²⁵ The GABA receptor subunit genes are deleted in both of the common deletion classes (I, II) so these genes do not account for differences seen among deletion-class groups but may contribute to the differences observed between deletion and non-deletion patients. No correlation between genotype and electroencephalographic pattern has emerged.³¹

Hypopigmentation occurs more frequently in AS deletion patients.⁵⁵ The (un-imprinted) *OCA2* gene located telomeric within the commonly deleted region is responsible for autosomal recessive *OCA2*. Individuals with AS and *OCA2* have been reported, the mechanism being a maternal chromosome deletion and paternal *OCA2* mutation.^{57,126} Semidominant behavior of the *OCA2* product (expression of a hypomorphic allele) has been offered to explain the hypopigmentation seen in AS deletion patients. However, hypopigmentation has been reported in other classes of AS patients, including siblings with a maternally inherited intragenic deletion of exons 8–16 of *UBE3A*, suggesting that *Ube3a* can alter pigmentation.¹²⁷ The melanocortin-1 receptor, which is downregulated in *Ube3a*-null mice, is a potential effector.⁵⁸

Status of clinical research

Clinical trials conducted thus far have produced negative results. Attempts to increase transcription from the paternal allele through the use of pro-methylation vitamin supplements did not result in any noticeable improvement.^{128,129} There is an ongoing randomized, placebo-controlled trial using levodopa/carbidopa to treat AS.¹³⁰ The rationale for this trial was based on the observations that levodopa was able to influence phosphorylation of CaMKII threonine residues in a rat model of Parkinson's disease;^{131,132} the finding of dopaminergic neuronal loss in AS mouse models;¹³³ and a report of two adults with AS and Parkinsonian symptoms who responded to levodopa.¹³⁴ There was a short open-label trial of minocycline treatment,¹³⁵ results of which have not yet been subjected to peer review. The rationale for minocycline was made based on the observation of elevated matrix metalloproteinase-9 activity in the hippocampi of AS mice (personal communication) and minocycline's ability to reduce matrix metalloproteinase-9 activity.

Status of basic research

Interrupting the AS pathophysiology may be undertaken at the level of gene transcription or at the point of protein interactions.¹³⁶ Mouse models for AS (the history of which was summarized recently¹³⁷), have been invaluable in exploring both of these avenues. The *Ube3a*^{m-p+} mouse has reduced brain size, ataxia, motor deficits, abnormal EEG, inducible seizures, and behavioral alterations.^{111,133} The AS mouse demonstrates impairments of context-dependent learning and memory and hippocampal LTP.¹¹⁰ Abnormalities of function in cerebellar Purkinje cells^{110,137,138} and nigrostriatal pathways^{133,139} have been reported. The behavioral phenotype of the *Ube3a*^{m-p+} mouse was recently characterized in a comprehensive manner,¹⁴⁰ which will assist greatly in the evaluation of future potential treatments.

Transcriptional upregulation has been approached by both gene therapy¹⁴¹ and pharmacologic intervention.¹⁴² Daily et al showed that direct injection of a recombinant adeno-associated viral vector carrying *Ube3a* into the hippocampi of adult AS mice could significantly improve associative learning (contextual fear conditioning).¹⁴¹ Huang and colleagues have demonstrated that the topoisomerase topotecan can un-silence the paternal *Ube3a* allele in cultured mouse primary cortical neurons, apparently by reducing transcription of *Ube3a-ats*.¹⁴² Recently the mechanism of inhibition of *Ube3a-ats* was shown to be mediated through RNA:DNA hybrid loop stabilization with the paternal snoRNA cluster.¹⁴³

Identifying the pathways in which *Ube3a* participates may lead to targeted intervention.¹³⁴ Studies have shown a role for CaMKII,^{113,114} neuregulin-ErbB4 signaling,¹¹⁷ EphB/Ephexin5 signaling,¹⁰⁵ and Arc (possibly mediated through brain-derived neurotrophic factor-induced TrkB-PSD-95¹⁴⁴) signaling.^{103,104} These observations point to a critical role for *Ube3a* in experience-dependent synaptic remodeling, possibly through more than one molecular pathway.

Conclusion

AS is a syndromic form of intellectual disability with a distinctive clinical presentation that can be best recognized by behavioral and performance characteristics. Molecular testing can diagnose most, if not all, cases. The causative protein, UBE3A, is critical for the processes of learning and memory through activity-dependent synaptic plasticity. Strides are being made in understanding the molecular pathogenesis, aided by mouse models that faithfully recapitulate the clinical syndrome.

Disclosure

The author declares no conflicts of interest in this work.

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