Applicability of genetic polymorphism analysis for the diagnosis of Angelman syndrome and the correlation between language difficulties and disease phenotype

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ABSTRACT. Angelman syndrome (AS) is a neurogenetic disorder caused by a defect in the expression of the maternally inherited ubiquitin protein ligase E3A (*UBE3A*) gene in chromosome 15. The most common genetic defects include maternal deletions in chromosome 15q11-13; however, paternal uniparental disomy and imprinting defects allow for the identification of mutations in *UBE3A* in 10% of patients with AS. The aim of this study was to validate the clinical features and genetic polymorphisms of AS, and to discuss the relationship between functional language lateralization and the arcuate fasciculus in the Broca's and Wernicke's areas. Six children with AS (mean age = 32.57 months) presenting characteristic behavioral patterns of AS (frequent laughter and happy demeanor, hand flapping, and hypermotor behavior) were recruited to this study. The patients underwent a clinical evaluation (clinical history, dysmorphological and neurological examinations, and psychological evaluations) and paraclinical investigations [genetic tests (fluorescence *in situ* hybridization and methylation polymerase chain

reaction), electroencephalogram, and magnetic resonance imaging]. We conclude that AS diagnosis cannot rely solely on genetic testing for polymorphisms in *UBE3A* and must consider its clinical characteristics. Moreover, functional language lateralization and the arcuate fasciculus in the Broca's and Wernicke's areas were found to be closely correlated. Therefore, *UBE3A* gene mutation analysis combined with comprehensive clinical evaluations may be suitable for the diagnosis of AS.

Key words: Angelman syndrome; Mutation analysis; Diagnosis; Language; Arcuate fasciculus

INTRODUCTION

Angelman syndrome (AS) is a neurodevelopmental disorder (Dagli et al., 2012), with clinical features including severe mental retardation, characteristic facial features (deep set eyes; pointed chin; wide, smiling mouth; and blonde hair/blue eyes, especially in patients with a microdeletion in the gene), a happy disposition, jerky movements, gait ataxia, and behavioral problems (Fiumara et al., 2010). Patients with AS also often exhibit seizures, microcephaly, and autistic features (Peters et al., 2004). Partial seizures are the most frequent seizure type observed in patients with AS, followed by atonic seizures and atypical absences. Electroencephalograms (EEGs) of children with AS always showed characteristic patterns of AS: slow background activity mixed with spikes, which arose even before the onset of epilepsy. Children studied using computed tomography (CT) and magnetic resonance imaging (MRI) have also been shown to present structural abnormalities such as ventricular enlargement, leukomalacia, cerebral atrophy, abnormal white-gray matter difference, diffuse cerebral lesions, and plagiocephaly (Duca et al., 2013). A prevalent clinical feature of AS is the absence of language and speech modalities (Williams et al., 2006).

The known genetic markers of AS include a maternal deletion in chromosome 15 (del[15q11-13]; occurring in 75-80% of patients), or a paternal uniparental disomy (UPD), intellectual disability (ID), and mutations in *UBE3A*. Most commercially available DNA methylation analyses cannot distinguish between AS resulting from a deletion, UPD, ID, and *UBE3A* mutation (Lossie et al., 2001). The abnormality is localized to *UBE3A*. The chromosomal defect is responsible for the dysfunction of the γ -amino butyric acid (GABA) receptor, as well as the disruption in the synthesis and secretion of GABA. The GABA receptor is a common channel facilitating the function of many drugs used in general anesthesia (Kim et al., 2010).

The arcuate fasciculus is a white matter pathway that provides reciprocal connections between the inferior frontal, parietal, and posterior temporal cortices, including the classical language regions, the Broca's and Wernicke's areas.

The aim of this study was to validate the general clinical features of AS and the AS-specific genetic polymorphism in *UBE3A*, and to discuss the relationship between functional language lateralization and the arcuate fasciculus in the Broca's and Wernicke's areas.

MATERIAL AND METHODS

Six patients with AS (mean age = 32.57 months, 4 females) were recruited to this study. This study was conducted in accordance with the declaration of Helsinki, and with

approval from the Ethics Committee of the Qingdao Women and Children's Hospital. Written informed consent was obtained from the families of all participants. All patients presented the characteristic behavioral phenotype of AS, such as frequent laughter and a happy demeanor, hand flapping, and hypermotor behavior. The clinical features of all patients, including the clinical history; results of general, dysmorphological, and neurological examinations; and psychological traits, including the developmental quotient (DQ) score and behavioral phenotype, of all patients were summarized. Additionally, the patients were subjected to paraclinical investigations such as biological testing, EEG, and neuroimaging (MRI). The seizure history in all patients, specifically the onset and type of seizure, was obtained. Genetic testing was conducted by the Jinan King Med Center clinical laboratory, and included fluorescence *in situ* hybridization (FISH) of the AS region in the gene with specific probes and methylation PCR analysis.

RESULTS

Patient characteristics

The clinical characteristics of the 6 patients with AS are presented in Table 1. All children presented a normal height, a flat occiput, and microcephaly, the latter of which has been highlighted as a frequent feature of AS (having been observed in > 80% of the cases) by the current Consensus Criteria for the Diagnosis of AS (Duca et al., 2013). The classical facial phenotype of AS (prognathia and a wide mouth) was also observed in all children. Hypopigmented skin and light hair and eye colors were also observed in > 83% of the cases. Ataxia was noted in 83% of the included patients. All children presented the characteristic behavioral phenotype of AS, such as frequent laughter (or a smiling disposition) and a happy demeanor. Excitability (in the form of hand flapping) and truncal hypotonia was observed in 67% of the cases during infancy. Although only 67% of the cases presented epileptic hallmarks, EEG abnormalities were observed in all children and persisted even when the seizures were controlled.

Table 1. Clinical characteristics of patients with Angelman syndrome.

Clinical features	Positive cases	Percentage (%)
Microcephaly	6	100
Flat occiput	6	100
Seizures	4	67
Abnormal EEG	6	100
Wide mouth	6	100
Hypopigmented skin, light hair, and eye color	5	83
Hyperactive extremity deep tendon reflexes	6	100
Frequent laughter/ smiling	6	100
Happy demeanor	6	100
Prognathia	6	100
Trunkal hypotonia during infancy	4	67
Gait ataxia	5	83
Excitability/ hand flapping	4	67

Neuropsychological evaluation

Developmental evaluations, performed using the revised Gesell Developmental Observation manual, assessed the global cognitive functioning, fine motor skills, language

functioning, and parent ratings of adaptive behavior and behavioral problems. The developmental characteristics of children with AS are presented in Table 2.

Table 2. Developmental evaluation scores of patients with Angelman syndrome.

Parameters	Patient number					
	1	2	3	4	5	6
Gender	Female	Male	Female	Female	Male	Female
Years (months)	25	31.5	41.5	33.4	29	35
Motor (DQ)	58	58	36	38	68	32
Fine motor skills (DQ)	51	58	36	31	48	30
Adaptive (DQ)	47	61	35	32	44	28
Personal-social (DQ)	47	66	34	32	44	25
Language comprehension (DQ)	47	63	35	38	55	31
Language expression (DQ)	30	34	28	23	26	21

DQ = developmental quotient. The evaluation of cognitive development (fine motor skills) in the AS patients revealed that 3 children (50% of the study population) had mild motor development delay, while the remaining presented severe delay. Adaptive and personal-social development was mildly, moderately, and severely delayed in 1 (17%), 2 (33%), and 3 (50% of the study subjects) children, respectively. Language comprehension development was mildly, moderately, and severely delayed in 2 children (33%), 1 (17%), and 3 (50% of the AS patients) children, respectively. Language expression was severely delayed in all patients. Moreover, the region of language expression was significantly different ($P < 0.05$).

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Seizures and EEG traces in patients with AS

Seizures were recorded in 67% of all included Patients with AS (Table 1), and were of varied types, including febrile, atypical absence, partial, myoclonic, and secondary generalized seizures. One patient had been previously diagnosed with epilepsy. The patients presented characteristic EEG patterns of rhythmic delta and theta mixing spikes over anterior, posterior, and generalized areas, as well as spikes and waves that were mainly distributed over the posterior area (Figure 1).

MRI results in patients with AS

All 6 patients underwent an MRI. The children showed different degrees of ventricular enlargement, cerebral atrophy, and white matter diminution. As shown in Figure 2, these structural abnormalities became more apparent with age.

Molecular genetic analysis

A genetic diagnosis of AS is generally performed by FISH analysis. Patients who

test negative are then re-tested with methylation PCR. FISH- and methylation PCR-negative patients are subjected to a *UBE3A* sequence analysis. However, it is impossible to detect large fragment gene deletions, repeat mutations, tiny mutations, and flanking intron gene sequences using these methods. Moreover, the overall cost of these tests would be prohibitive for an average household. In this study, one of the patients presented a loss of the SNRPN maternal 15q11-13 segment and another presented a single parent (father) diploidy. A *UBE3A* c. 2567_2568d AA p. p (857 f s Glu) hybrid mutation was observed in a third patient. The remaining three were FISH- and methylation PCR-negative and refused a *UBE3A* sequence analysis. Despite this, the clinical features of the latter three patients were sufficient for the diagnosis of AS.

Figure 1. Characteristic electroencephalogram (EEG) pattern shown by patients with Angelman syndrome (AS). The EEG presents slow background activity mixed with spikes.

Figure 2. T2- weighted magnetic resonance imaging (MRI T2W) of a patient with Angelman syndrome (AS) showing white matter diminution, ventricular enlargement, and cerebral atrophy.

DISCUSSION

AS is a rare and severe neurodevelopmental disorder with a complex clinical picture. AS is associated with characteristic facial (deep-set eyes; pointed chin; wide, smiling mouth) and behavioral features, ataxia, and developmental delays. The behavioral features of AS include a happy demeanor, easily-provoked laughter, a short attention span, hypermotor behavior, sleep disturbances with a reduced need for sleep, and an affinity for water (Horsler and Oliver, 2006; Pelc et al., 2008; Williams, 2010). Although AS causes difficulties during infancy because of feeding problems and general irritability, most children with AS have

a happy disposition (Williams, 2010). However, children with AS are extremely excitable. Moreover, although paroxysms of laughter are said to occur in Patients with AS, this laughter is not truly “unprovoked,” given that an inciting event can usually be identified. However, laughter is frequently excessive or inappropriate with respect to the triggering stimulus (Bird, 2014). Approximately 80-95% of patients with AS experience epileptic seizures (Thibert et al., 2013). The mean age of onset of these seizures is 1.9 years, with approximately 86% of all Patients with AS under 3 years of age being diagnosed with epilepsy (Park et al., 2012). Many seizure types, both generalized and focal, have been reported, including atypical absence, myoclonic, atonic, and generalized tonic-clonic (GTC) seizures. The frequency of occurrence of the GTC and myoclonic seizures is the highest in Patients with AS, followed by atonic and atypical absence seizures. GTC seizures showed a tendency to occur with fevers. Additionally, status epilepticus (SE) with a predominant number of myoclonic seizures has been reported to occur in 21% of all patients (Park et al., 2012). The seizure activity can, however, be attenuated in adolescents (Thibert et al., 2009, 2013). The EEG of Patients with AS presents a very characteristic pattern (slow background activity mixed with spikes) (Vendrame et al., 2012), which can sometimes be useful in the diagnosis of AS. In fact, a previous report by Park et al. (2012) characterized the EEG pattern in 86% of all Patients with AS as follows: a typical AS pattern characterized by runs of high-amplitude slow waves that were more prominent in the frontal region, mixed with spike and slow wave multifocal activity. Interictal EEG patterns were, however, not related to the type of initial seizures, with EEG abnormalities persisting in controlled seizures (Park et al., 2012). The above-described characteristic phenotype allows for a relatively easy clinical diagnosis of AS, even in younger children (aged 6 months - 3 years), in direct contrast to the opinion that AS is difficult to diagnose. All patients included in this study presented the characteristic facial features and behavioral phenotype of AS. Additionally, all 6 children presented microcephaly, a flat occiput, a wide mouth, hyperactive extremity deep tendon reflexes, frequent laughter (or a smiling disposition), a happy demeanor, prognathia, and abnormal EEG patterns. A total of 83% of the children also had hypopigmented skin, light hair and eye color, and gait ataxia, and 67% of the patients experienced seizures and truncal hypotonia during infancy, or excitability (characterized by hand flapping). All patients were clinically diagnosed with AS.

Although AS is characterized by aberrant facial features and behavior, developmental delays in AS are usually evident within the first year of life. Moreover, the uptake of gross motor and fine motor skills, receptive language, expressive language, and social skills in Patients with AS is delayed. Individuals with AS have been reported to experience a plateau in their developmental level, reaching an upper limit of 24 to 30 months; moreover, cognitive performance in these patients is usually severely functionally impaired (Bird, 2014). Movement disturbances, abnormalities of muscle 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 (Bird, 2014). Language development is significantly impaired in patients with AS. Most individuals lack speech entirely, although a few individuals have small single-word vocabularies (Bird, 2014); however, the use of phrases is very rare. Commonly used developmental assessment tools cannot be used to comprehensively analyze individuals with AS, because of the combination of deficits expressed by these patients; additionally, we have observed these tools to underestimate the abilities of children with AS. Consistent with this report, all patients exhibited a developmental delay in our study. Expressive language delay is common in syndromes associated with intellectual

disabilities; AS is unusual in that all individuals have extremely poor expressive language skills that are out of proportion to their overall level of intellectual disability and receptive language skills. It is notable that overall, the most consistent finding is the uniformly poor expressive language skill in all participants regardless of their molecular sub-class. Since the single common molecular mechanism in all participants is the lack of UBE3A protein in parts of the brain where *UBE3A* is imprinted, a hypothesis can be developed that UBE3A may be essential in the development of expressive language skills (Gentile et al., 2010).

Patients with AS display another common feature, in addition to language-related disabilities. The degree of lower head circumference, ventricular enlargement, cerebral atrophy, and white matter diminution was different in all children included in this study. Peters et al. (2004) also reported reduced myelination in the white matter of the brain. DTI tractography studies in participants with AS have also shown abnormalities in the arcuate fasciculus, inferior fronto-occipital fasciculus, inferior longitudinal fasciculus, uncinate fasciculus, corticospinal tracts, and corpus callosum (Peters et al., 2011). The arcuate fasciculus is a white matter tract that connects the language comprehension region of the temporal lobe with the speech-generating region of the frontal lobe. Protein degradation during development through ubiquitination is a common mechanism for the regulation of axon guidance molecule levels (Hu et al., 1997; Myat et al., 2002). Evidence from genetic studies analyzing the development of several other fiber tracts, including the corticospinal tract (Rünker et al., 2008), midbrain dopaminergic axons (Stuebner et al., 2010), and sensory axons (Steinel and Whittington, 2009), indicate that the axon guidance mechanisms play an important role in the proper formation of these tracts. UBE3A is principally expressed in the neurons of the hippocampus, cerebellum, and cortex, and is involved in the ubiquitin proteasome system, which is the primary mechanism by which protein degradation is regulated within the cell. Ubiquitination-based degradation of proteins during development is a common mechanism for the regulation of axon guidance molecule levels (Dindot et al., 2008). This could be due to a potential problem with axon guidance during brain development, which could be attributed to the loss of *UBE3A* gene expression. Previous studies have identified various clinical differences between the molecular classes of AS. Patients expressing a deletion in this gene tend to be shorter and lighter than the general population, whereas those with UPD or imprinting defects tend to be taller and heavier; patients with mutations in *UBE3A* tend to show variable growth (Tan et al., 2011). Younger Patients with AS with mutations in *UBE3A* scored higher in tests of cognition, gross motor and fine motor skills, and receptive language, than patients with a deletion in this gene (Cafferkey et al., 2014). This could also mean that alterations in the mechanisms responsible for regulating axon guidance in part affect the development of the arcuate fasciculus, particularly the orientation of its fibers.

In conclusion, AS is a syndromic form of intellectual disability with a distinctive clinical presentation. It is best recognized by behavioral and performance characteristics. Mutations in *UBE3A* could be analyzed for the diagnosis of AS. However, the analysis of genetic polymorphisms does not preclude the need for a comprehensive analysis: because of the varied characteristics of AS, a clinical diagnosis is of greater significance. Functional language lateralization and the arcuate fasciculus in the Broca's and Wernicke's areas are said to be closely related.

Conflicts of interest

The authors declare no conflicts of interest.

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