Angelman syndrome: clinical findings and follow-up data of 14 patients

Bülent Kara[1], Birsen Karaman[2], Meral Özmen[3], Rasim Özgür Rosti[2], Mine Çalışkan[3] Hülya Kayserili[2], Seher Başaran[2]

[1]Department of Pediatrics, Kocaeli University Faculty of Medicine, Kocaeli, and Departments of [2]Medical Genetics, and [3]Pediatrics, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey

SUMMARY: Kara B, Karaman B, Özmen M, Rosti RÖ, Çalışkan M, Kayserili H, Başaran S. Angelman syndrome: clinical findings and follow-up data of 14 patients. Turk J Pediatr 2008; 50: 137-142.

The diagnosis of Angelman syndrome (AS) is based on the clinical features, behavior, EEG findings, and genetic abnormalities. The physical, clinical and behavioral aspects appear to attributable to localized central nervous system (CNS) dysfunction of the ubiquitin ligase gene, UBE3A, located at 15q11.2. The features of AS frequently become apparent at 1-4 years of age, and the average age at diagnosis is 6 years.

Angelman syndrome was considered in the differential diagnosis of 30 patients who were referred to the Medical Genetics Department of İstanbul Medical Faculty between 1995 and 2005. The diagnosis was confirmed in 14 patients (8 female, 6 male) by detecting the presence of deletion through the use of fluorescence in situ hybridization (FISH) technique in all, while high-resolution banding technique (HRBT) detected only seven of the deletions. The patients' ages at the time of diagnosis ranged from 2 to 12 (mean 4.10±2.59) years.

We report here on 14 patients with definite diagnosis of AS who displayed the characteristic clinical features of the syndrome and additional findings not previously reported, along with the follow-up data concerning neuromotor development and seizures.

Key words: Angelman syndrome, dysmorphism, epilepsy, mental retardation, behavioral abnormalities.

Angelman syndrome (AS) is a neurogenetic syndrome affecting children[1,2]. It was first described in 1965 by Harry Angelman, an English pediatrician[3]. The prevalence of AS is estimated to be around 1/10,000-1/20,000, and among individuals with severe developmental delay, as 0%, 1.3%, 1.4%, and 4.8%, in different studies[1-4]. AS is characterized by severe mental retardation, inappropriate laughter, happy mood, ataxic gait, jerky/puppet-like movements and minimal or absent speech[5]. Dysmorphic craniofacial features tend to become more prominent with age and include postnatal onset micro-brachycephaly, mid-facial hypoplasia, deep-set eyes, macrostomia and prominent mandible[5]. Epileptic seizures occur in about 80% of patients[4].The EEG is usually more abnormal than clinically expected, but it can also be normal in individuals with genetically proven AS[1]. AS is a clinical diagnosis that can be confirmed by genetic testing in about 80-85% of the cases[4]. The physical, clinical and behavioral aspects appear to be attributable to localized central nervous system (CNS) dysfunction of the ubiquitin ligase gene, UBE3A, located at 15q11.2.

We report here on 14 patients with definite diagnosis of AS who displayed the characteristic clinical features of the syndrome and additional findings not previously reported, along with the follow-up data concerning neuromotor development and seizures.

Material and Methods

Thirty patients were referred to the Medical Genetics Department of İstanbul Medical Faculty between 1995 and 2005 due to unidentified

etiology of severe mental retardation, severe speech deficit, dysmorphic features, and epileptic seizures or abnormal EEG findings, and AS was suspected in the differential diagnosis. All patients were evaluated by a clinical geneticist. Metabolic screening tests were performed in all and cranial magnetic resonance imaging (MRI) in 29.

Cytogenetic analysis was performed by high-resolution banding technique (HRBT) using thymidine on all of the patients' blood lymphocytes. Twenty metaphases were analyzed for each individual. Fluorescent in situ hybridization (FISH) studies were carried out with SNRPN probe (Cytocell). Hybridization and application were performed according to the manufacturers' protocols. DAPI (4', 6-diamidino-2-phenyl-indole), FITC, and Rhodamine fluorescence signals were detected using specific filter combinations (Pinkel #1, Chroma Technology).

Results

The diagnosis of AS was confirmed in 14 patients (8 female, 6 male) who had preliminary diagnosis of AS by detecting the presence of deletion through the use of FISH technique in all, while HRBT detected only seven of the deletions (Table I). The patients' ages at the time of diagnosis ranged from 2 to 12 years (mean 4.10±2.59 years). Gender, age, age and head circumference at diagnosis, and cytogenetic analysis (HRBT and FISH) results of patients are shown in Table I. All patients showed severe developmental delay, severe speech deficit or absent speech, movement and gait problems and behavioral abnormalities. Six of them (42.8%) were ambulatory, 2 (15%) could walk with support, and 6 could not walk; 11 of them (78.5%) had absence of speech, and 2 (14.2%) were able to speak a few meaningful words. The gross motor and language development of the patients are summarized in Table I. Dysmorphic facial features, neurologic and behavioral abnormalities and pathologic EEG findings consistent with the diagnosis of AS according to the criteria of Williams et al.[6] are shown in Table II. Additional clinical findings observed in our patients are summarized in Table III. Dysplastic and/or simple ear and helical abnormalities (42.8%), clinodactyly (35.7%), flat philtrum (35.7%), pes equinovarus deformity (21.4%), midfacial hypoplasia (21.4%), retro-micrognathia (21.4%), narrow high-arched palate (28.5%) and finger pads (28.5%) were the frequently associated physical findings in our group of patients.

All patients had seizures and were on antiepileptic therapy. Seizures were completely controlled in 4 patients, and partially controlled in 10. The

Table I. Gender, Age, Age and Head Circumference at Diagnosis, Time of Gross Motor and Speech Development, and Cytogenetic Analysis (HRBT and FISH) Results of 14 Patients with Angelman Syndrome

No.	Gender	Age (years)	Age at diagnosis (years)	Head control	Unsupported sitting	Walking	Speech (single words)		FISH	HRBT
1	Female	7	3	46.5	?	18 months	4.5 years	None	+	–
2	Male	12	5	50.2	?	6 months	None	None	+	–
3	Female	20	12	50.5	1.5 years	5 years	None	None	+	–
4	Male	3 6/12	3 3/12	46.5	?	6 months	None	None	+	–
5	Female	4	2 6/12	44	9 months	2 years	None	3 years	+	–
6	Female	6	5	48	5 months	2 years	2.5 years	None	+	–
7	Female	12	5	47	3.5 months	2.5 years	None	2 years	+	+
8	Female	5	3	47	2 months	7 months	18 months	None	+	+
9	Male	3 6/12	3	47	3 months	11 months	2.5 years	None	+	+
10	Female	6	6	45.5	1 month	14 months	2.5 years	None	+	+
11	Male	2 9/12	2 4/12	45	?	6 months	2 8/12 years	None	+	+
12	Male	2	2	46.5	12 months	18 months	None	None	+	+
13	Female	6.5	3	47	1 month	11 months	None	None	+	+
14	Male	2 4/12	2 4/12	47	3 months	11 months	None	None	+	+

HRBT: High–resolution banding technique. FISH: Fluorescent in situ hybridization.

Table II. Frequency of Features in our Series According to Angelman Syndrome Diagnostic Criteria of Williams et al. (1995)

A-Consistent features	Frequency
Developmental delay, functionally severe	14/14 (100%)
Speech impairment, none or minimal	14/14 (100%)
Movement or balance disorder	14/14 (100%)
Behavioral abnormalities	14/14 (100%)
B-Frequent features	
Absolute microcephaly	10/14 (71.4%)
Relative microcephaly	4/14 (28.5%)
Seizures	14/14 (100%)
Abnormal EEG	14/14 (100%)
C-Associated features	
Sleep disturbance	12/14 (85.7%)
Attraction to or fascination with water	11/14 (78.5%)
Wide mouth, wide-spaced teeth	10/14 (71.4%)
Hypopigmented skin, light hair and eye color	8/14 (57.1%)
Frequent drooling	7/14 (50%)
Hyperactive lower extremity deep tendon reflexes	7/14 (50%)
Increased sensitivity to heat	7/14 (50%)
Strabismus	6/14 (42.8%)
Uplifted, flexed arm position during ambulation	5/14 (35.7%)
Feeding problems during infancy	5/14 (35.7%)
Flat occiput	4/14 (28.5%)
Protruding tongue	4/14 (28.5%)
Prognathia	3/14 (21.4%)

Table III. Frequency of Additional Anomalies Observed in our Series Not Listed in the Consensus Criteria (Williams et al.)

Feature	Frequency
General	
Thin skin	1/14 (7.1%)
Dry plantar skin	1/14 (7.1%)
Face	
Dysplastic and/or simple ear; helix abnormalities	6/14 (42.8%)
Flat philtrum	5/14 (35.7%)
Narrow high–arched palate	4/14 (28.5%)
Midfacial hypoplasia	3/14 (21.4%)
Retro–micrognathia	3/14 (21.4%)
Long eyelashes	2/14 (14.2%)
Epicanthus	2/14 (14.2%)
Synophrys	1/14 (7.1%)
Narrow forehead	1/14 (7.1%)
Laterally sparse eyebrows	1/14 (7.1%)
Short philtrum	1/14 (7.1%)
Preauricular pit	1/14 (7.1%)
Trunk	
Pectus excavatum	1/14 (7.1%)
Extremities	
Clinodactyly	5/14 (35.7%)
Finger pads	4/14 (28.5%)
Pes equinovarus deformity	3/14 (21.4%)
Unclear palmar and plantar creases	3/14 (21.4%)
Simian crease	3/14 (21.4%)
Joint hyperextensibility	2/14 (14.2%)
Hockey crease	1/14 (7.1%)
Hyper–flexibility of first and fifth fingers	1/14 (7.1%)
Cubitus valgus	1/14 (7.1%)
Plantar crease	1/14 (7.1%)
Genitourinary system	
Cryptorchidism	2/6 (33.3%)
Genital hypoplasia	1/14 (7.1%)

onset of seizures, seizure type at onset, seizure-free period at follow-up, age at last seizure, and the recent antiepileptic therapy administered are summarized in Table IV.

Cranial MRI findings were normal in 11 patients and in 2 showed minimal cerebral atrophy. Cranial imaging was not performed in 1 patient. Metabolic screening tests revealed normal results in all patients. Family history was uneventful except for one with an epilepsy history.

Discussion

Angelman syndrome (AS) is a genetically-determined developmental disorder caused by deletion of the maternally inherited chromosome 15q11-13 (75% of cases), paternal uniparental chromosome 15 disomy (2-3% of cases), methylation imprinting mutation (2-3% of cases), and UBE3A mutation (2-3% of cases)[7]. In the remaining 15-20% of patients, the genetic mechanism is still unknown. DNA methylation testing is a reliable screening test for deletions, uniparental disomy (UPD) or imprinting center (IC) defects, but it does not distinguish which of the three mechanisms is operative[1,4]. To determine the underlying mechanism, the next step is to perform chromosome 15 FISH analysis to detect 15q11.2-15q13 deletions. If there is no deletion, additional genetic testing is necessary to determine if either UPD or IC defects are present[1]. If the initial DNA methylation test is normal, UBE3A mutations may be the possible genetic mechanism, since these mutations have no effect on the DNA methylation pattern of the 15q11.2-15q13 region. Even though UBE3A mutation testing reveals normal results, the remaining group of patients could still be affected by AS, thus belonging to 10-15% of cases with unidentified etiology[1]. In our series, the diagnosis of AS was confirmed by only HRBT and FISH in 14 of 30 (47%) patients with the clinical characteristics of AS. It would well be possible that the number of AS patients may increase if the other three known genetic mechanisms operative for AS had been tested in our remaining 16 patients.

UBE3A was identified as the AS gene, located within the 15q11-13 region, in 1997 by two different groups[8,9]. It produces a protein called the E6-associated protein (E6AP), which acts as a cellular ubiquitin ligase enzyme[1]. In

Table IV. Onset of Seizures, Seizure Type at Onset, Seizure–Free Period at Follow–Up, Age at Last Seizure, EEG Abnormalities at Diagnosis, and Recent Antiepileptic Therapy in 14 Patients with Angelman Syndrome

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13	Case 14
Age at first visit (years)	3	5	12	3 3/12	2.5 5	5	3	3	6	2 4/12	2	3	2 4/12	
Age at seizure onset (years)	2 3/12	1/12	1	1	6/12 4	1 3/12	1 3/12	11/12	1	2	3/12	1	10/12	
Seizure type at onset	Afebrile GT	Afebrile GT	Afebrile GTC	Febrile ?	Febrile GTC Partial	Afebrile ?	Febrile ?	Febrile GTC	Spasm GTC	Afebrile Myoclonic	Febrile GTC	Afebrile ?	Afebrile	Febrile
Seizure–free period at follow–up (years)	4/12	4	1	2 6/12	1	6/12	6/12	?	2/12	4/12	4/12	1/12	5/12	1 6/12
Total seizures	Multiple	Multiple	Multiple	1	4	6	Multiple	?	Multiple	Multiple	4	Multiple	2	1
EEG abnormality at diagnosis	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Recent antiepileptic therapy	VPA CLBZ	VPA	VPA CLNZ	VPA	PB	VPA	VPA PB	VPA CLNZ	VPA CLNZ	VPA	VPA	VPA	LMTG CLNZ	VPA

GTC: Generalized tonic–clonic. GT: Generalized tonic. VPA: Valproic acid. LMTG: Lamotrigine. CLNZ: Clonazepam. CLBZ: Clobazam. PB: Phenobarbital.

certain regions of the normal brain, UBE3A is expressed only from the maternal allele and its expression in the AS brain with 15q11.1-15q13 deletion is only about 10% that of normal[10]. This phenomenon of monoallelic or single chromosome regional expression is termed genomic imprinting, and AS is a typical example[1,11]. There is limited correlation between the clinical severity of AS and its type of genetic mechanism. Individuals with the large chromosome deletions are more likely to have seizures and microcephaly and are more likely to have skin, eye, and hair hypopigmentation[1]. Those with uniparental disomy are more likely to have no seizures, normal head circumference, and better cognitive functioning, although severe to profound impairment is still present. Those with UBE3A and IC defects are more likely to have moderate clinical severity between the former and latter mechanisms stated above[1]. Chromosomal deletion was the only genetic mechanism detected in our patients, and as such, when evaluating the clinical features of our patients, they should be compared only with the deleted AS cases reported previously.

Severe speech deficit, severe mental retardation, behavioral abnormalities and movement problems are ubiquitous in AS, while other features, such as microcephaly or seizures, may be absent[1]. The diagnosis of AS is primarily clinical and can be confirmed by laboratory tests[12]. However, its diagnosis remains difficult in infants who do not present the typical features[13]. If the child is less than 12 months of age, tremulous movements, ataxia or severe lack of speech may not be apparent, and likewise seizures may not have occurred yet[1]. The facial features and physical examination may generally appear normal, and development during the first six months of life is not apparently delayed, although protruding tongue, strabismus, brisk deep tendon reflexes and apparent happy demeanor may be present in this period[14]. As the child with AS grows, the definite diagnosis may be possible with speech being essentially absent and gait anomalies becoming evident due to severe jerkiness and ataxia[1]. Typical dysmorphic features evolve over the first five years[4]. They are socially outgoing, quite hyper-motoric and are moving forward developmentally[1]. Pediatricians often first encounter AS while consulting on an infant with the problem of developmental delay, microcephaly or seizures. AS should be on the differential diagnosis list of any child with microcephaly, seizures, typical behavioral problems and gait abnormalities. In our group of patients, age at diagnosis was 4.10±2.59 years, some two years earlier than reported data. Early confirmed diagnosis of AS can help to avoid unnecessary investigations. Family planning issues can also be discussed more in advance.

A consensus for diagnostic criteria was established in 1995, and updated in 2005 by Williams et al.[6]. They appear indicative in clinical practice even if the diagnosis can not be excluded when they are not uniformly present[5]. There are three parts of the consensus criteria. All the features of part A are consistent with AS; thus, patients should have all of these features for the diagnosis of AS. Features of part B and part C are not necessary for the diagnosis. Features of part C contain many minor physical abnormalities, sleep disturbance and attraction to water. In our series, we observed some physical findings not listed in the consensus criteria. Dysplastic and/or simple ear and helix abnormalities, clinodactyly, midfacial hypoplasia, retro-micrognathia, flat philtrum, finger pads and pes equinovarus deformity were the frequently associated minor anomalies in our series. These new findings may be included in part C of the consensus criteria, if further studies support our findings.

The prevalence of EEG abnormalities is about 80% in AS patients[15]. EEG abnormalities are much more prominent in AS patients with a deletion (97-100%), although normal EEG background activity was reported in 72.2% of AS patients with UPD, IC defects and UBE3A mutations[16,17]. EEG abnormalities were observed in all our AS patients.

The prevalence of epilepsy is 80-90% in AS patients[1,2,4]. The age at seizure onset is usually between 1 and 2 years, but seizures can occur in infants less than 1 year old or older than 3 years[2]. Seizures may be difficult to control, especially in early childhood, and may decrease or cease by the time the patient is in their mid-teens or early adulthood[2,4]. However, some authors report good control with classic antiepileptic drugs for generalized and partial seizures, but not in non-convulsive

status epilepticus and atypical absences[5,14]. The most effective antiepileptic drugs are valproate in combination with clonazepam or other benzodiazepines, whereas carbamazepine sometimes has an adverse effect[4]. Experience with new antiepileptic drugs is limited. All of our patients had a history of epileptic seizures. Epileptic seizures were under complete control in 4 patients and partial control in 10 patients with classic antiepileptic drugs.

In conclusion, the prevalence of AS in patients with severe mental retardation, speech deficit and epilepsy is not rare. In infants with typical EEG findings, developmental delay and behavioral abnormalities, with or without seizures, the diagnosis of AS should be considered and genetic testing performed. Early diagnosis of the syndrome is important for genetic counseling, early therapeutic intervention of epileptic seizures, especially nonconvulsive status epilepticus, and avoidance of over-treatment for EEG abnormalities[5,14].

REFERENCES

1. Williams CA. Neurological aspects of the Angelman syndrome. Brain Dev 2005; 27: 88-94.
2. Rubin DI, Patterson MC, Westmoreland BF, Klass DW. Angelman's syndrome: clinical and electroencephalographic findings. Electroenceph Clin Neurophysiol 1997; 102: 299-302.
3. Angelman H. Puppet children: a report on three cases. Dev Med Child Neurol 1965; 7: 681-688.
4. Laan L, Haeringen A, Brouwer OF. Angelman syndrome: a review of clinical and genetic aspects. Clin Neurol Neurosurg 1999; 101: 161-170.
5. Buoni S, Grosso S, Pucci L, Fois A. Diagnosis of Angelman syndrome: clinical and EEG criteria. Brain Dev 1999; 21: 296-302.
6. Williams CA, Beaudet AL, Clayton-Smith J, et al. Angelman syndrome 2005: updated consensus for diagnostic criteria. Am J Med Genet 2006; 140: 413-418.
7. Miano S, Bruni O, Leuzzi V, Elia M, Verrillo E, Ferri R. Sleep polygraphy in Angelman syndrome. Clin Neurophysiol 2004; 115: 938-945.
8. Kishino T, Lalande M, Wagstaff J. UBE3A/E6-AP mutations cause Angelman syndrome. Nat Genet 1997; 15: 70-73.
9. Matsuura T, Sutcliffe JS, Fang P, et al. De novo truncating mutations in E6-AP ubiquitin-protein ligase gene (UBE3A) in Angelman syndrome. Nat Genet 1997; 15: 74-77.
10. Rougeulle C, Glatt H, Lalande M. The Angelman syndrome candidate gene, UBE3A/E6-AP, is imprinted in brain. Nat Genet 1997; 17: 14-15.
11. Jiang Y, Tsai TF, Bressler J, Beaudet AL. Imprinting in Angelman and Prader-Willi syndromes. Curr Op Genet Develop 1998; 8: 334-342.
12. Wang PJ, Hou JW, Sue WC, Lee WT. Electroclinical characteristics of seizures- comparing Prader-Willi syndrome with Angelman syndrome. Brain Dev 2005; 27: 101-107.
13. Hou JW, Wang PJ, Wang TR. Angelman syndrome assessed by neurological and molecular cytogenetic investigations. Pediatr Neurol 1997; 16: 17-22.
14. Ohtsuka Y, Kobayashi K, Yoshinaga H, et al. Relationship between severity of epilepsy and developmental outcome in Angelman syndrome. Brain Dev 2005; 27: 95-100.
15. Laan LA, Vein AA. Angelman syndrome: is there a characteristic EEG? Brain Dev 2005; 27: 80-87.
16. Laan LA, Renier WO, Arts WF, et al. Evolution of epilepsy and EEG findings in Angelman syndrome. Epilepsia 1997; 38: 195-199.
17. Minassian BA, DeLorey TM, Olsen RW, et al. Angelman syndrome: correlations between epilepsy phenotypes and genotypes. Ann Neurol 1998; 43: 485-493.