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Clinical Evaluation Followed by Molecular Diagnosis in a Multiplex Pedigree of Angelman Syndrome: A Case Report

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Abstract

Angelman syndrome (AS) is a neurodevelopmental disorder characterized by severe developmental delay or intellectual disability caused by either disruption of the maternal *UBE3A* gene or deletion in the maternal 15q11-q13. Diagnosis of AS in two siblings with normal four-generation history was confirmed through a staged genetic evaluation, G-banding karyotype, Fluorescence *in situ* Hybridization analysis, and methylation pattern in 15q11-q13. Despite detecting impaired methylation patterns in the region of interest, distinguishing the imprinting defects from uniparental disomy (UPD) was not feasible since we did not have access to the parents' sample. However, regarding the low risk of AS recurrence within families with UPD in the literature and the healthy family history, mosaicism of cryptic imprinting center deletion in the mother's germline should be the most probable cause of AS recurrence in our cases.

Keywords: Angelman syndrome; Germline mosaicism; Molecular diagnosis; Familial recurrence; Mental retardation.

Introduction

Angelman syndrome (AS; OMIM #105830) is a rare neuro-genetic disorder associated with delayed development and mental retardation with 1 in 10,000–24,000 births [1, 2]. This condition involves distinctive behavioral phenotypes in addition to structural and functional abnormalities, including hyperactivity, microcephaly, intellectual disability, epilepsy, impairment of speech ataxia, abnormal EEG, tongue protrusion, and sleeping disorder [2, 3].

15q11-q13 region has a parent of origin-specific DNA methylation patterns, which is related to two different disorders, Prader–Willi syndrome (PWS) and Angelman Syndrome. AS individuals fail to inherit a normal active maternal copy of the ubiquitin protein ligase E3A (*UBE3A*) gene mapped to chromosome 15q11-q13 and expressed exclusively from the maternal chromosome in the brain [4, 5]. Apart from 10% of AS cases with unknown underlying mechanisms, loss of function of the maternal *UBE3A* can result from a maternal allele mutation (11%), paternal uniparental disomy (UPD) of chromosome 15 (3-7%), and aberrant imprinting due to either deletion of the maternally

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inherited chromosomal region (~65-75%), or a defect in imprinting mechanism (1–3%) resulting in the deficiency of ubiquitin protein ligase E3A in neurons [6]. These individuals have a biparental inheritance of chromosome 15, but the maternal copy lacks methylation of the *SNRPN* promoter/exon 1 region, leading to the expression of *SNHG14* and suppression of maternal *UBE3A* transcripts [2]. Imprinting defects can result from either a deletion in the imprinting center (IC) or epimutation without any modification in its DNA sequences which is more common. More than 40% of affected patients in the latter group demonstrate imprinting defects only in a mosaic form which is a post-fertilization event [2, 7].

Genetic testing is necessary to confirm the clinical diagnosis and assess the recurrence risk in AS families. Herein, we described the recurrence of AS in two siblings with mental and developmental delays and no familial history of this disorder. Furthermore, its etiology was examined through cytological and molecular techniques.

Materials and Methods

An Iranian family with two 17- and 14-year-old siblings with severe intellectual and developmental retardation were referred to us for confirmation of Angelman syndrome (AS) diagnosis. Precise clinical and physical examination and complete family history were accomplished. 5–10 mL of peripheral blood from both siblings was collected after written consent.

G-banding karyotype analysis of peripheral lymphocytes was performed to detect the common 3-4 Mb deletion in 15q11-13 or other cytogenetically visible chromosome rearrangements (i.e., inversion or translocation). First, 450 µl of the peripheral blood cells were cultured for 72 hours in 4 ml RPMI, 1/5 ml FBS, and 70 µl of PHA. Afterward, 100 µl colcemid was added to the culture medium and incubated for 30 min at 37 ºC. Subsequently, 7 ml of hypotonic potassium chloride solution was added and incubated for 30 min at 37 ºC. In the following step, cells were fixed with methanol: acetic acid (3:1). Finally, slides were prepared from the metaphase chromosome in a controlled temperature environment with a humidity of 52%. Using the Giemsa trypsin banding (GTG) method, at least 20 metaphase spreads were banded and analyzed based on the International System for Human Cytogenetic Nomenclature (ISCN) 2016 criteria [8].

Fluorescence *in situ* Hybridization (FISH) analysis of metaphase spreads and interphase nuclei was performed with a specific probe for *SNRPN/IC* locus at 15q11.2 and a control locus at 15qter (Cytocell Inc.) according to the manufacturer's instruction. This probe covers the 170 Kbp region proximal to *SNRPN* and its first exon. Preparation of probes, *in situ* hybridization, scoring of signals, and capturing the images were performed as previously described [9]. This 170 Kbp probe could detect small deletions of the IC, including *SNRPN* locus, present in 80-90% of patients with an imprinting defect [1].

Table 1. Oligonucleotide sequences of the primers used for PCR assay

Gene	Primer sequence
SNRPN	Forward: 5'-AGGTCATTCCGGTGAGGGAGG-3' Reverse: 5'-CCCCTCCTCTAGACAGCA ATGAT-3'
CFTR	Forward: 5'-CTATGACCCGGA TAACAAGGAGGAGC-3' Reverse: 5'-AGAATATATGTGCCATGGGGCCTGTG-3'

For investigating the methylation status, 500 ng of genomic DNA was digested by *NotI* and *McrBC* enzymes (20 units in 20 µl and 10 units in 10 µl reaction, respectively) with 12 hours incubation at 37 ºC which was followed by an inactivation process at 65 ºC for 20 minutes. Although the *McrBC* enzyme only cuts the methylated restriction site, the *NotI* enzyme cannot cut the restriction site if it is methylated. In the next step, PCR amplification was performed on 100 ng DNA with specific primers for the *SNRPN* locus and the control region in the *CFTR* gene (Table 1) using a PCR kit (Yekta Tajhiz Azama, Iran) according to the manufacturer's instruction. PCR products were subsequently analyzed using agarose gel electrophoresis.

Results

As shown in Figure 1, the family medical history of four-generation was normal. Mother had an uneventful gestational course for both children, which were normal at birth. The girl started having seizures at four months old, repeated every 2 to 3 months. Both siblings had delayed motor development, and the girl started walking at age 15, but the boy could not stand up unaided until ten years of age. Siblings are urinary incontinent, fascinated with water, and insensitive to pain. They cannot speak or contain saliva. They laugh instead of crying with uplifted hands, flapping motions, open mouth, and protruding tongue. They also have a happy disposition with unprovoked episodes of laughter and staring spells.

The girl has mild microcephaly, scoliosis, gait

ataxia, no limb tremor, and weak reflexes in the lower limbs. Except for little pubic and axillary hair, she does not have other secondary sexual characteristics such as developed breasts and a menstrual cycle. Dilation of the ventricles and the visible temporal horn of the lateral ventricle were observed in the brain CT scan. The boy has a flat face with blepharitis, a broad nasal bridge, a bulbous nose tip, wide-spaced teeth, and a narrow forehead. He also has ataxia with limb trembling, slight deep tendon reflex, and pes planus.

As shown in Figure 1, the family medical history of four-generation was normal. Both siblings showed normal chromosomes in their G-banding karyotype analysis of peripheral lymphocyte karyotypes (Figure 2). FISH analysis for *SNRPN*/*IC* locus also had normal results (Figure 3). In the investigation of the methylation status, it was established that the *SNRPN* locus was digested by NotI but McrBC, as there was no

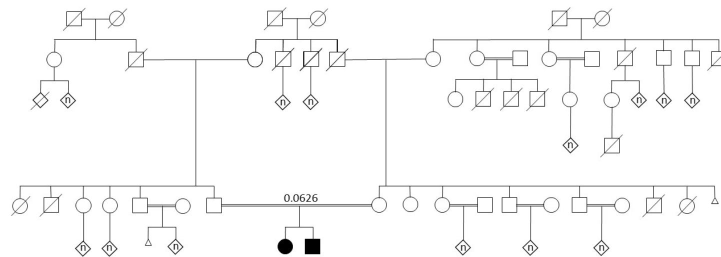

Figure 1. A four-generation pedigree of the reported family.

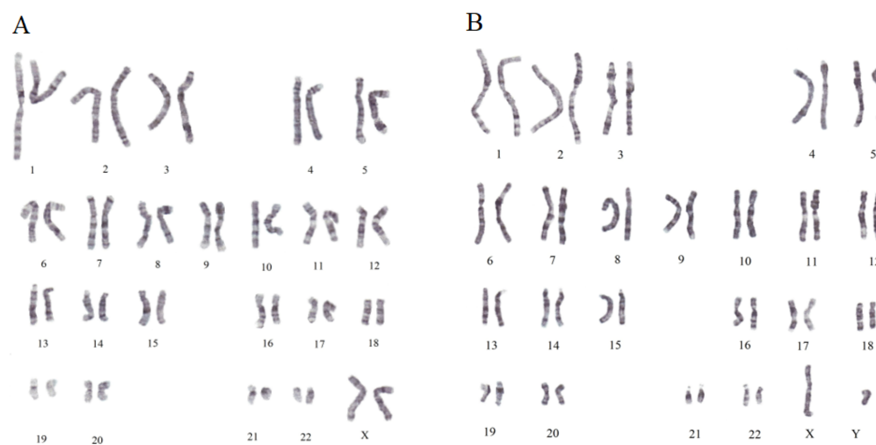

Figure 2. High-resolution G-banding karyotype from female (A) and male (B) siblings with normal chromosome count and structure.

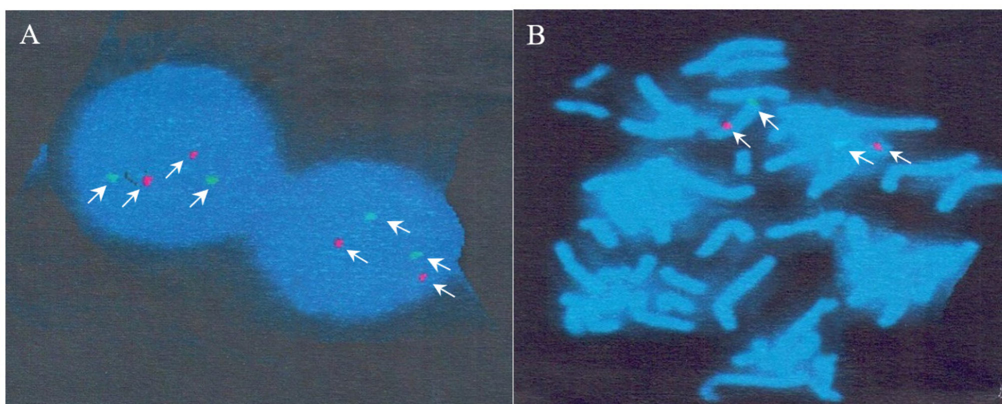

Figure 3. FISH analysis of hybridization of the specific probe for *SNRPN/IC* locus (Texas red) and 15q sub-telomeric probe (FITC) on the interphase (A) and metaphase (B) nuclei. FISH signals show normal *SNRPN* locus on chromosome 15.

amplification of treated DNA with *NotI* by specific primers for *the SNRPN* locus.

Discussion

The defective methylation pattern in 15q11-q13 derived from either the absence of the maternal allele or the maternal allele with the wrong paternal epigenotype is a common observation in AS cases [10]. Genetic counseling and recurrence risk assessment is a challenge in this disorder as most of the reason leading to AS is due to a de-novo deletion in 15q11-q13 with low recurrence risk. Also, the recurrence risk is 50% in cases without any identifiable UPD or large deletion; this might result from mutations in the UBE3A gene or IC in the maternal chromosome [6]. We excluded any rearrangement or detectable deletion via karyotyping and FISH analysis for the *SNRPN* locus [2, 11, 12]. The impaired methylation patterns of 15q11-q13 confirmed the diagnosis of AS. Our cases could result from either imprinting defects or UPD; however, as we did not have access to the parents' sample, we did not accomplish further tests to distinguish imprinting defects from UPD.

Paternal UPD accounts for 3-7% of AS cases, resulting from nondisjunction of maternal chromosome 15 followed by duplication of paternal counterpart in post-zygotic stages, which could be associated with Robertsonian translocation [1, 2]. there is no report of AS recurrence in cases with UPD and normal chromosomes in the literature. Thus, there is a valid rationale to suppose that if the karyotype of the mother is normal for chromosome 15, the risk of recurrence is very low for the family. However, it should not be considered zero (<1%) [1, 2, 13]. On the other hand, there is a correlation between UPD-derived AS and obesity which is not an observed phenotype in our cases [2].

Imprinting defects could cause by either epimutation or genetic modification of IC. Sporadic pre- or post-zygotic epimutation, the primary underlying mechanism (~90%) of imprinting defect in AS, has shown a low risk of recurrence [2, 10, 14]. Imprinting defect with no changes in IC structure, derived from either the imprinting maintenance failure in the early embryonic stage of the mother's cells or an error in the establishment of imprinting pattern in the maternal germline during oogenesis, has shown a low risk of recurrence within the family. However, Reis *et al.* reported the recurrence of AS in siblings due to the epimutation. These patients showed paternal methylation imprint in the maternal chromosome, which could result from a subtle rearrangement or mutation within or near the critical PWS/AS region [15].

Regarding the imprinting defects due to the genetic changes, the failure of switching the parental imprinting pattern caused by an IC microdeletion is reported in several AS cases [2]. Around 10-20% of AS cases with imprinting defects are due to 6-200 kb microdeletion, including a deletion in IC (*de novo* or inherited) which could not be detected by FISH or array CGH analysis [1, 10]. Few cases with IC deletions are identified as *de novo,* or the mother is reported to have germline mosaicism for this deletion [2]. While the recurrence risk is not increased in the former condition, it is more common in the latter [11, 16]. On the other hand, in nearly every case with a familial history of AS, the IC deletion is an inherited mutation that could be transmitted through the germline of the male carrier from the previous generation with no phenotype manifestation. Still, when it is transmitted through the germline of the female with a normal karyotype, it disrupts the establishment of maternal imprinting in offspring [1, 2]. In other cases, the mother acquires the IC deletion following a spontaneous mutation on her paternal chromosome 15 [1]. Therefore, the risk of having a child with AS for a female carrier of an IC deletion is 50%, as normal FISH result could not rule out small deletions or mutations of the IC [2]. In this context, the recurrence of AS due to the IC deletion was observed in affected siblings. However, there is no report of recurrence in the family of patients with non-IC deletion imprinting defect AS in the literature, except for a report of recurrent cases with IC rearrangement [1, 14, 17]. Buiting *et al.* reported a 1 Mbp inversion within the 15q11-q13 region leading to the disruption of the IC, which was transmitted through the male germline silently to the mother of two siblings with AS [17].

Altogether, we could rule out UPD since there is no report of AS recurrence in Proband families with UPD and normal karyotype. Besides, inherited familial IC deletion seems unlikely since the history of the sizeable four-generation family shows no evidence of familial IC deletion, which could be transmitted through the maternal germline to siblings. Therefore, mosaicism of the IC deletion in the mother's germline is our cases' most probable cause of AS recurrence.

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