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Original article

Uniparental disomy and imprinting defects in Japanese patients with Angelman syndrome☆

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Abstract

We examined 54 patients with deletion-negative Angelman syndrome (AS) using DNA methylation testing and microsatellite polymorphism analysis, and identified three patients with paternal uniparental disomy (UPD) and seven patients with imprinting defects (ID). The three patients with UPD were shown to have paternal isodisomy 15, which we hypothesized to have arisen from duplication of chromosome 15. Two of the patients with ID were siblings and carried microdeletions of the imprinting center (IC), while the remaining five patients had no evidence of deletions and represented sporadic cases. Two of the three patients with UPD and two of the seven patients with ID had not developed seizures. The only patients displaying microcephaly were those with ID who had microdeletions at the IC. These data support the previous findings that indicate that patients with UPD and ID may have a milder phenotype of AS.

Keywords: Angelman syndrome; Genomic imprinting; DNA methylation; DNA diagnosis; Uniparental disomy; Imprinting defect

1. Introduction

Angelman syndrome (AS) is a disorder related to genomic imprinting with complex underlying genetic mechanism [1,2]. The *UBE3A* gene, which is imprinted and expressed exclusively from the maternal allele in certain areas of the brain, has been implicated in AS. Five molecular classes (class I: deletion; class II: uniparental disomy (UPD); class III: imprinting defects (ID); class IV: *UBE3A* mutation; class V: unknown etiology) lead to loss of function of maternally expressed *UBE3A* and result in the AS phenotype [3]. Therefore, systematic molecular genetic tests are required to establish the diagnosis and molecular class of AS [2]. Since fluorescence in situ hybridization (FISH), which can detect class I deletions, has been the only commercially available test in Japan, many patients with deletion-negative AS have not received definitive genetic diagnosis. Therefore, limited information is available in Japan on patients with deletion-negative AS. We performed systematic molecular genetic tests for Japanese patients with deletion-negative AS. In this study, we report the molecular and clinical characteristics of patients with AS with UPD or ID in Japan.

2. Materials and methods

2.1. Patients

Fifty-four patients with deletion-negative AS were subjected to molecular analyses. The 54 patients included 46 sporadic patients and eight familial patients. The eight familial patients consisted of sibling pairs from four different families. Clinical information was collected through questionnaires and the diagnosis of AS was given based on the criteria of Williams et al. [4]. Deletions in 15q11-q13 were excluded by commercial karyotyping using

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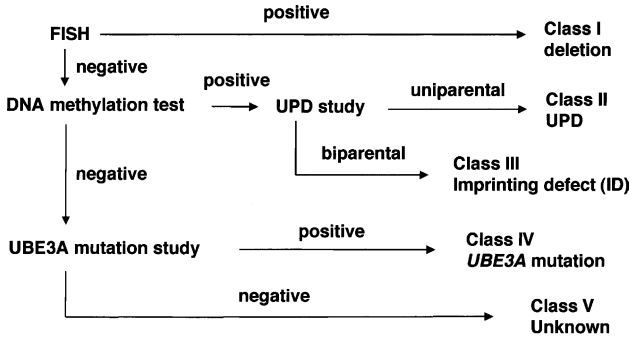

Fig. 1. Molecular diagnostic algorithm for Angelman syndrome adopted in this study.

FISH with the *D15S10* probe. Informed consent for this study was obtained from the parents.

2.2. Molecular analyses

An adopted algorithm for molecular analysis is shown in Fig. 1. If DNA methylation testing shows an AS specific pattern, polymorphism analysis is performed to distinguish UPD (class II) from ID (class III). ID cases are subsequently subjected to Southern blot hybridization to detect microdeletions of the imprinting center (IC). If DNA methylation tests are normal, a *UBE3A* mutation study is performed to distinguish between classes IV and V cases. Classes IV and V will be reported elsewhere.

Genomic DNA was extracted from peripheral blood leukocytes by standard techniques. A PCR-based DNA methylation test for *SNURF–SNRPN* was performed using the bisulfite method [5]. Chemical treatment of DNA with bisulfite converts cytosine (C) to uracil (U), unless C is methylated. Converted U is recognized as thymine (T) during PCR reaction, and thus methylation status of C is transformed to sequence difference (C or T). The CpG island of *SNURF–SNRPN* that is located in vicinity of IC of 15q11-q13 is completely methylated on a maternally derived inactive allele, while completely unmethylated on a paternally derived active allele. Therefore, the methylated maternal allele and the unmethylated paternal allele can specifically be amplified and distinguished by bisulfite treatment and subsequent PCR [5].

Microsatellite polymorphism analyses using PCR revealed five highly polymorphic loci located in 15q11-q13 (*D15S11, D15S128, D15S817, D15S97, GABRB3*), one locus in 15q13-qter (*ACTC*), and one locus in 11q13.5 (*D11S527*). Allelotypes were examined on the ABI PRISM 310 machine, using GeneScan software (Applied Biosystems, Foster City, CA).

Microdeletion studies were performed using Southern blot hybridization with a series of probes spanning the IC [6,7].

Table 1
Summary of molecular diagnosis in 54 patients with Angelman syndrome

Test/analysis	Results	No. of patients (%)
DNA methyl-	Negative	44 (81)
ation test	Positive	10 (19)
Polymorphism	Uniparental isodisomy 15	3 (6)
study	Uniparental heterodisomy 15	0 (0)
	Biparental disomy 15	7 (13)
IC deletion	IC deleted	1 sib-pair
analysis	IC present	5

3. Results

3.1. Molecular genetic classification of Japanese AS patients

From 54 patients with deletion-negative AS, we identified three patients with paternal UPD and seven patients with ID (Table 1). The three patients with UPD were all sporadic cases and showed homozygosity at all loci examined on chromosome 15. They were classified as having paternal isodisomy for chromosome 15 (isoUPD). Two patients with ID were siblings and demonstrated microdeletion of the IC. The mother of these patients was shown to be mosaic for the microdeletion; detailed molecular data of this family (AS-J) have been reported previously [7]. The remaining five patients with ID were sporadic cases that showed no evidence of deletion at the IC.

3.2. Clinical characteristics

We compared the phenotype of the patients with UPD and ID. Although some clinical features appeared to be characteristic of either UPD or ID (Table 2), these patients did not exhibit typical AS phenotypes. Two of the three patients with UPD did not experience seizures, and one patient had no detectable EEG abnormality. Two of the seven patients with non-IC-deleted ID had no seizures, although none of these patients had normal EEGs. None of the patients with UPD displayed microcephaly. Microcephaly was also absent in patients with non-IC-deleted ID, but it was associated with the siblings who carried deletions of the IC. Consistent clinical features between both groups

Table 2
Major clinical findings in each molecular class of AS

	Patients with		
	UPD ($n=3$) ID	non-IC-del ($n=5$) ID	IC-del ($n=2$)
Current age, average (years)	12.6	15.6	15
Walking age, average (years)	2.9	2.5	ND
Obesity (>2 SD)	1/3	2/5	ND
Microcephaly	0/2	0/5	2/2
Seizure	1/3	3/5	2/2
EEG abnormality	2/3	4/4	2/2
Hypopigmentation	2/2	2/5	0/2

of patients included severe speech delay, typical facies, and characteristic behaviors. Notably, one of the patients with UPD and two of the patients with ID were obese, and the patient with UPD was initially misdiagnosed as having Prader–Willi syndrome (PWS).

4. Discussion

In this study, three of 54 patients with deletion-negative AS (6%) had UPD, and seven of 54 patients with deletion-negative AS (13%) had ID. The frequency of UPD and ID in this study was generally in agreement with previous reports [1]. A significant number of the patients with deletion-negative AS can be classified using a combination of DNA methylation testing and microsatellite polymorphism analysis, and this classification has important implication for genetic counseling. Recurrent risks of ID are associated with IC microdeletions [8]. In this study, two of the seven patients with ID were siblings that had microdeletion of the IC and were familial cases, while the other five patients had no deletions and represented sporadic cases. While it therefore makes sense to screen all patients with ID for IC microdeletions, current detection methods are laborious and time consuming and subtle mutations may still be missed. These factors make recurrent risk assessment for ID a challenging task. The three patients with UPD identified in this study were shown to have paternal uniparental isodisomy at all tested loci. These findings are consistent with duplication of chromosome 15 in early embryogenesis as the major mechanism of uniparental disomy. However, non-disjunction during paternal meiosis II cannot be excluded [9].

Patients with UPD and ID have a relatively mild phenotype of AS [3,10,11]. For patients with AS with typical deletions, seizures are seen in approximately 90% of cases. In this study, two of three patients with UPD and two of seven patients with ID did not experience seizures. Furthermore, microcephaly was not a feature of patients with UPD or non-IC-deleted ID. These findings are in agreement with the observation that there is a less severe phenotype with UPD or ID. In fact, one patient with UPD was only mildly ataxic and demonstrated a developmental quotient (DQ) of 47 at 5 years of age, despite the acquisition of only one word. Another patient with non-IC deleted ID also displayed a less severe phenotype with very mild ataxia and no seizures, but she had no meaningful words. There is an association of obesity with patient with AS with UPD or ID [9,12] and in our study two of seven patients with ID and one of three patients with UPD were obese, the latter initially being misdiagnosed as having PWS. Although, our series of patients demonstrated a less severe phenotype, all showed severe speech delays and characteristic behaviors. No patients demonstrated more than a couple of meaningful words, and it may be that the core phenotype of AS is speech and/or expressive language dysfunction, along with characteristic behaviors.

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