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ARTICLE

Phenotypic variability in Angelman syndrome: comparison among different deletion classes and between deletion and UPD subjects

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Angelman syndrome (AS) can result from either a 15q11–q13 deletion (del), paternal uniparental disomy (UPD), imprinting, or *UBE3A* mutations. Here, we describe the phenotypic and behavioral variability detected in 49 patients with different classes of deletions and nine patients with UPD. Diagnosis was made by methylation pattern analysis of exon 1 of the *SNRPN-SNURF* gene and by microsatellite profiling of loci within and outside the 15q11–q13 region. There were no major phenotypic differences between the two main classes (BP1–BP3; BP2–BP3) of AS deletion patients, except for the absence of vocalization, more prevalent in patients with BP1–BP3 deletions, and for the age of sitting without support, which was lower in patients with BP2–BP3 deletions. Our data suggest that gene deletions (*NIPA1, NIPA2, CYF1P1, GCP5*) mapped to the region between breakpoints BP1 and BP2 may be involved in the severity of speech impairment, since all BP1–BP3 deletion patients showed complete absence of vocalization, while 38.1% of the BP2–BP3 deletion patients were able to pronounce syllabic sounds, with doubtful meaning. Compared to UPD patients, deletion patients presented a higher incidence of swallowing disorders (73.9% del × 22.2% UPD) and hypotonia (73.3% del × 28.57% UPD). In addition, children with UPD showed better physical growth, fewer or no seizures, a lower incidence of microcephaly, less ataxia and higher cognitive skills. As a consequence of their milder or less typical phenotype, AS may remain undiagnosed, leading to an overall underdiagnosis of the disease.

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Introduction

Angelman syndrome (AS)[1] comprises developmental delay, severe mental retardation, absent speech, seizures, ataxia, outbursts of laughter, microcephaly, brachycephaly, macrostomia, and prognathism. Gait is described as wide-based, with arms held flexed and upheld at the elbows. AS is caused by the loss of expression of maternal imprinted gene(s) mapped to the chromosome region 15q11–q13.

AS and Prader–Willi syndrome (PWS – neonatal hypotonia, poor sucking, delayed psychomotor development, hyperphagia, obesity, short stature in adolescents and adults, small hands and feet, hypogonadism, mild to moderate mental retardation, temper tantrums, obsessive-compulsive mannerisms) were the first examples in hu-

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mans of genomic imprinting or the differential expression of genetic material, depending on the parent of origin. While AS results from the loss of expression of a maternal gene (*UBE3A*), PWS results from the loss of expression of paternal imprinted genes mapped to the chromosome region 15q11–q13.

Four different mechanisms can lead to the AS phenotype: two-thirds of AS cases have a maternal deletion within 15q11–q13; paternal uniparental disomy of chromosome 15 (UPD15) is detected in 2–3%; approximately, 2% have a mutation in the imprinting center and, in about 8%, mutations in the *UBE3A* gene are found.

The chromosome region 15q11–q13 is meiotically unstable, with an unusual variety of cytogenetic rearrangements, including the AS and PWS deletions, duplications and triplications, inv dup(15) marker chromosomes, inversions, and balanced or unbalanced translocations. In 95% of PWS/AS patients with a deletion, two main classes of deletions are found.[2–4] Class I patients show breakpoints at BP1 (proximal) and BP3 (distal), while Class II patients present breakpoints at BP2 (proximal) and BP3 (distal). The remaining 5% have the distal breakpoint at BP4. Another breakpoint, BP5, has been reported only in inv dup(15) marker chromosomes[5,6] and in some cases of interstitial duplications and triplications of chromosome 15q11–q13.[7–9] Recent studies have shown that the deletions in 15q11–q13 are mediated by nonallelic homologous recombination between low-copy repeats (duplicons), which map to the common deletion breakpoint regions.[3,4]

In general, patients with AS resulting from large chromosome deletions appear to be more severely affected than patients belonging to the other genetic classes. Several authors have shown that AS patients with UPD have milder phenotypes than patients with deletions.[10–13] They pointed out that children with UPD have a better physical growth, frequently with weight above the 75th centile, fewer or no seizures, less ataxia, and better cognitive skills. Hypopigmentation is more frequent among patients with deletions, since the nonimprinted gene responsible for pigmentation (P) that causes type II oculocutaneous albinism[14] is located in the distal portion of 15q11–q13.

When compared to deletion AS subjects, patients with imprinting defects are less likely to have microcephaly, hypopigmentation, or seizures, and show better growth, motor milestones, and communication skills. Obesity is relatively common in this group.[15]

Patients with *UBE3A* mutations have abilities that fall somewhere in between those of the deletion and the UPD group. They frequently have seizures and microcephaly, but hypopigmentation is not detected.[16] Motor and communication skills are better than in the deletion group. Lossie *et al*[17] pointed out that in this group the frequency of obesity is particularly high as the patients get older.

Herein we report the proximal and distal breakpoints of chromosome segment 15q11–q13 detected in 46 deletion AS patients (13 BP1–BP3 – Class I; 22 BP2–BP3 – Class II; two BP2–BP4 – Class III, one BP2–BP5 – Class IV, eight inconclusive) and describe the phenotypic and behavioral variability detected among 35 patients with different classes of deletions (13 Class I; 22 Class II) and among 49 patients with deletions and nine patients with UPD.

Materials and methods

Patients

Investigation of breakpoints was carried out on 46 AS deletion patients, and phenotypic and behavioral studies on 58 patients (34 female and 24 male subjects with ages ranging from 1 year and 3 months to 30 years and 5 months): 49 with a 15q11–q13 deletion and nine with UPD. These patients were diagnosed in our laboratory from July 1996 through July 2003.

Most patients were referred for genetic testing for AS by physicians of the Neurology Department and the Children's Institute of the University of São Paulo School of Medicine and were examined by at least one of the authors, following a standard protocol that included evaluation of physical and behavioral characteristics. Informed consent was obtained for all patients from a legal guardian.

In all, 21 of the patients with a deletion and four with UPD were previously reported in Fridman *et al*,[13] and another eight of the nine UPD patients presented here were described in Fridman *et al*.[18]

Genetic studies

DNA was extracted from peripheral blood leukocytes by standard procedures. Diagnosis was established by methylation pattern analysis of the PWS/AS region. The DNA was modified by bisulfite treatment, and the *SNURF-SNRPN* exon 1 amplified by PCR.[19] A characteristic AS pattern is recognized by the presence of the 221 bp paternal band only (data not shown).

Three markers within the critical region 15q11–q13 (*D15S11, D15S113* and *GABRB3*)[20] and at least one marker outside this region (*D15S984, D15S131, D15S117, D15S115* or *CYP19*) were studied in patients and their parents, to distinguish between deletion and paternal uniparental disomy (data not shown).

Investigation of the extent of the deletion was performed by microsatellite analysis with markers mapped to the segment 15q11–q14 (*D15S11, D15S113, GABRB3, D15S1002, D15S1048, D15S1019, D15S165, D15S1031, D15S1043, D15S1010*) in patients and their parents.

Chromosome studies of patients were performed on peripheral blood lymphocytes, using the GTG-banding technique to investigate structural and numerical alterations.

Statistical analysis

Absolute frequencies of phenotypic characteristics in subgroups of patients were compared in 2×2 contingency tables, using Fisher's exact test that generates the exact probabilities corresponding to the null hypothesis of nonassociation. Quantitative measurements and counts were contrasted using Mann–Whitney's nonparametric test.

Results

All patients presented normal karyotypes. Of 46 AS deletion patients analyzed with *D15S541/D15S542* markers, 41 were informative for at least one of the two markers. Overall, in 34.46% (14/41), a *S541/542* deletion was found, indicating that BP1 was the proximal deletion breakpoint. The other 65.54% (27/41) were heterozygous at *S541/542*, indicating that BP2 was the proximal breakpoint. Of 43 informative cases, 40 (93.02%) presented the distal breakpoint at BP3 (proximal to *D15S1048/1019*); 4.66% (2/43) at BP4 (between *D15S1019* and *D15S165*), and 2.32% (1/43) at BP5 (between *D15S1031* and *D15S1010*). In summary, we found five breakpoint regions in AS deletion patients: BP1, BP2, BP3, BP4, and BP5. A total of 38 patients presented informative results for proximal and distal breakpoints concomitantly: 34.21% (13) belonging to Class I (BP1–BP3), 57.9% (22) to Class II (BP2–BP3), 5.26% (two) to Class III (BP2–BP4), and 2.63% (one) to Class IV (BP2–BP5).

Table 1 shows the frequency of the clinical and behavioral findings among patients with different classes of deletions (13 patients with deletions at BP1–BP3, 22 BP2–BP3, two BP2–BP4, one BP2–BP5). Table 2 shows the frequency of the clinical and behavioral characteristics of the 49 deletion and nine UPD patients.

Discussion

Comparison of behavioral and clinical findings among AS deletion patients with different classes of deletion

Our deletion patients had the same breakpoints already described,[2–4] with the exception of the distal breakpoint BP5, found in a single patient. A distal BP5 breakpoint had previously been detected only in large inv dup(15) chromosomes[5,6] and in some cases of interstitial duplications and triplications of chromosome 15q11–q13.[7–9]

All deletion patients presented a clinical phenotype typical of AS: developmental delay, severe mental retardation, macrostomia, outbursts of laughter, and ataxic gait. Class II deletion patients showed a better performance than Class I subjects in two developmental areas: sitting without support and vocalization. Sitting without support was achieved, on the average, at 16 months by Class II patients and at 19 months by Class I patients. Vocalization was more articulated in Class II, in which 38.1% of patients were able to pronounce syllabic sounds of doubtful meaning. Class I patients could only utter unarticulated sounds. No statistical differences could be demonstrated with respect to the age of neck support and independent gait, but Class II patients seemed to have a slightly better motor development (Table 1).

Class I patients also showed a higher incidence of hypotonia (84.6% in Class I $\times$ 61.1% in Class II), microcephaly (75% in Class I $\times$ 55% in Class II); seizures (present in 92.3% Class I $\times$ 85% Class II) and lower ability of communication by gestures (60% in Class I $\times$ 84.6% in Class II), although no statistical significance could be demonstrated.

The present study was able to establish a correlation between two different major deletion classes and the AS phenotype. Recently, four genes (*NIPA1, NIPA2, CYF1P1, GCP5*) were mapped to the region between breakpoints BP1 and BP2, but their function is still unknown.[21] Rainier *et al*[22] reported a dominant negative mutation in the *NIPA1* gene in a kindred with autosomal dominant hereditary spastic paraplegia linked to the SPG6 locus. The fact that Class I deletion patients with PWS and AS do not exhibit progressive spastic paraplegia indicates that *NIPA1* haploinsufficiency does not cause this disease.

Our data suggest that deletions of the genes mapped to the region between BP1 and BP2 might be involved in speech impairment and delayed acquisition of developmental abilities, since all BP1–BP3 deletion patients showed complete absence of vocalization, while 38.1% of the BP2–BP3 deletion patients were able to pronounce syllabic sounds, and the developmental delay was more severe in AS patients with BP1–BP3 than with BP2–BP3 deletions.

In a recent study, Butler *et al*[23] found that PWS subjects with Class I deletions have a more severe phenotype than those with Class II deletions, including self-injurious behavior, deficits in adaptive behavior (including motor skills), obsessive–compulsive behavior, and difficulties with reading, mathematics skills, and visual-motor integration.

Comparison of behavioral and clinical findings between AS deletion patients and paternal UPD patients

Our previous report on 21 AS deletion patients and four paternal UPD patients[13] stated that UPD patients use to be diagnosed later than deletion patients, mainly because the phenotypic and behavioral traits are more subtle in UPD children. Microcephaly and complete absence of speech were more frequent among deletion patients; UPD patients usually walked earlier and had seizures later than deletion patients. In the present study, we also observed an older age at diagnosis for UPD (average 9 years) than for deletion patients (average 5 years and 8 months). This was also

Table 1 Phenotypic characteristics of AS patients according to deletion class

	Class I deletion BP1–BP3 (13 patients)	*Class II deletion BP2–BP3 (22 patients)*	*Class III deletion BP2–BP4*		*Class IV deletion BP2–BP5*	*P*	
			AS20	*AS37*	*AS8*	*1*	*2*
Age at diagnosis (years)	$5^{4/12}$	$6^{1/12}$	3	$1^{5/12}$	4	0.984	
Maternal age (years)	28	$27^{1/12}$	30	16	28	0.711	
Paternal age (years)	$34^{6/12}$	$29^{11/12}$	31	22	27	0.159	
Swallowing difficulties	42.85 (3/7)	75% (9/12)	0	+	0		0.326
Hypotonia	84.6% (11/13)	61.1% (11/18)	–	+	+		0.237
Birth weight (average – g)	2997	2949.5	3750	2760	3110	0.841	
Birth height (average – cm)	48.4	49.2	51	43	49	0.337	
Neck support (years)	9/12	6/12	4/12	4/12	4/12	0.484	
Sitting without support (years)	**$1^{7/12}$**	**$1^{4/12}$**	8/12	–	–	**0.044**	
Independent gait (years)	$5^{2/12}$	$4^{1/12}$	–	–	–	0.211	
Absent speech	**100% (12/12)**	**61.9% (13/21)**	+	+	+		**0.030**
Developmental delay	100% (13/13)	100% (21/21)	+	+	+		1.000
Weight (centile)							
<25	40% (4/10)	43.75% (7/16)					1.000
25–75	40% (4/10)	37.50% (6/16)	×		×		1.000
>75	20% (2/10)	18.75% (3/16)		×			1.000
Height (centile)				?			
<25	42.85% (3/7)	41.2% (7/17)					1.000
25–75	42.85% (3/7)	41.2% (7/17)	×		×		1.000
>75	14.3 (1/7)	17.6% (3/17)					1.000
Microcephaly	75% (9/12)	55% (11/20)	+	–	–		0.538
Occipital groove	85.7% (6/7)	77.8% (7/9)	+	+	0		1.000
Macrostomia	100% (13/13)	100% (20/20)	+	+	+		1.000
Protruding tongue	77.8% (7/9)	66.7% (12/18)	+	+	–		0.676
Wide-spaced teeth	81.8% (9/11)	94.1% (16/17)	+	0	–		0.543
Severe mental retardation	100% (13/13)	100% (22/22)	+	+	+		1.000
Seizures (presence)	92.3% (12/13)	85% (17/20)	+	+	+		0.638
Seizures (age of onset) (years)	$1^{7/12}$	$1^{9/12}$	1	7/12	2	0.734	
(Range)	$4/12–2^{7/12}$	8/12–3					
Laughter outbursts	100% (13/13)	100% (21/21)	+	+	+		1.000
Ataxic gait	100% (8/8)	100% (13/13)	0	0	0		1.000
Hyperactivity	88.9% (8/9)	100% (15/15)	+	0	–		0.375
Capacity of communication	60% (3/5)	84.6% (11/13)	–	0	–		0.533
Sleep disturbance	80% (8/10)	80% (12/15)	+	+	–		1.000
Frequent drooling	100% (8/8)	94.5% (17/18)	+	0	+		1.000
Hyperphagia	1 patient	3 patients	–	0	–		1.000

P: Results of statistical tests for differences between Class I and Class II deletion patients.
1: Mann–Whitney's test.
2: Fisher's exact test.
Significant results ($P<0.05$) are indicated in bold.

reported by Bottani *et al*,[10] Gillenssen-Kaesbach *et al*,[11] and Smith *et al*.[12]

We did not detect any significant difference between maternal and paternal ages of UPD and deletion AS patients (Table 2), although the origin of the syndrome in UPD individuals usually depends on nondisjunction events that are associated with increased maternal age. In AS, most paternal UPD15 seem to be postzygotic events.[24,18]

In the present study, in addition to the differences described previously,[13] deletion patients presented a higher incidence of swallowing disorders ($73.9 \times 22.2\%$) and hypotonia ($73.3 \times 28.57\%$) than UPD patients.

Other features associated with the clinical diagnosis of AS, such as brachycephaly, occipital groove, macrostomia, wide-spaced teeth, outbursts of laughter, hyperactivity, sleep disturbance and frequent drooling, presented a higher incidence among deletion patients, suggesting that these patients have a more severe and more typical phenotype, although these differences did not reach statistical significance (Table 2). Haploinsufficiency of genes localized in the deleted chromosome segment is probably responsible for the more severe phenotypic and behavioral characteristics of deletion patients, as compared to patients with UPD, imprinting mutations, and *UBE3A* mutations.

The fact that UPD patients have a milder or less typical phenotype suggests that AS may be underdiagnosed. Based on our study, we recommend that developmental delay, severe mental retardation, speech impairment, happy

Table 2 Phenotypic characteristics of AS patients with deletions and UPD

	Deletion (49 patients)	*UPD (9 patients)*	*P* 1	2
Age at diagnosis (years)	$5^{8/12}$	9	0.087	
(Range)	$1^{3/12}$–$30^{5/12}$	$2^{7/12}$–21		
	29F; 20M	5F; 4M		
Maternal age (years)	$27^{8/12}$	$29^{5/12}$	0.562	
(Range)	16–39	23–40		
Paternal age (years)	$32^{1/12}$	$33^{9/12}$	0.497	
(Range)	20–52	25–47		
Birth weight (average – g)	2981	3328	0.055	
Birth height (average – cm)	49	48.5	0.447	
Swallowing difficulties	**73.9% (17/23)**	**22.22% (2/9)**		**0.015**
Hypotonia	**73.33% (33/45)**	**28.57% (2/7)**		**0.031**
Neck support (years)	7/12	4/12	0.059	
Sitting without support (years)	$1^{5/12}$	1	0.184	
Independent gait (years)	$4^{4/12}$	3	0.136	
Absent speech	91.5% (43/47)	66.6% (6/9)		0.074
Developmental delay	100% (49/49)	100% (9/9)		1.000
Weight (centile)				
<25	**35% (14/41)**	**0/9**		**0.047**
25–75	47.5% (19/41)	44.4% (4/9)		1.000
>75	**17.5% (8/41)**	**55.5% (5/9)**		**0.040**
Height (centile)				
<25	33.3% (12/36)	22.2% (2/9)		0.698
25–75	52.8% (19/36)	33.3% (3/9)		0.727
>75	13.9% (5/36)	44.5% (4/9)		0.063
OFC (centile)				
<50	**84.78% (39/46)**	**44.44% (4/9)**		**0.017**
50–75	13.04% (6/46)	22.2% (2/9)		0.604
75–98	2.17% (1/46)	11.1% (1/9)		0.303
>98	0/46	22.2% (2/9)		0.024
Microcephaly	**54.35% (25/46)**	**11.1% (1/9)**		**0.027**
Occipital groove	73.9% (17/23)	50% (2/4)		0.558
Macrostomia	100% (47/47)	88.8% (8/9)		0.161
Protruding tongue	70% (28/40)	66.6% (6/9)		1.000
Wide-spaced teeth	87.2% (34/39)	62.5% (5/8)		0.123
Severe mental retardation	100% (49/49)	100% (9/9)		1.000
Seizures (presence)	**89.4% (42/47)**	**44.4% (4/9)**		**0.006**
Seizures (age of onset) (years)	**$1^{7/12}$**	**$6^{4/12}$**	**0.002**	
(Range)	2/12–4	$1^{6/12}$–13		
Laughter outbursts	95.8% (46/48)	77.7% (7/9)		0.113
Ataxic gait	93.1% (27/29)	100% (8/8)		1.000
Hyperactivity	94.3% (33/35)	80% (4/5)		0.337
Capacity of communication	73.1% (19/26)	57% (3/4)		1.000
Sleep disturbance	80.5% (29/36)	71.4% (5/7)		0.624
Frequent drooling	96.9% (32/33)	87.5% (7/8)		0.356
Hyperphagia	8.1% (4/49)	33.3% (2/6)		0.123
Skin picking	—	60% (3/5)		—

P: 1: Mann–Whitney's test.
2: Fisher's exact test.
Significant results ($P<0.05$) are indicated in bold.

demeanor, with or without the presence of seizures, should be considered as minimal criteria for a *SNURF-SNRPN* exon 1 methylation assay test.

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