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ARTICLE

Phenotype–genotype correlation in 20 deletion and 20 non-deletion Angelman syndrome patients

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Angelman syndrome (AS) is a neurodevelopmental disorder caused by the absence of a maternal contribution to chromosome 15q11–q13. There are four classes of AS according to molecular or cytogenetic status: maternal microdeletion of 15q11–q13 (approximately 70% of AS patients); uniparental disomy (UPD); defects in a putative imprinting centre (IM); the fourth includes 20–30% of AS individuals with biparental inheritance and a normal pattern of allelic methylation in 15q11–q13. Mutations of UBE3A have recently been identified as causing AS in the latter group. Few studies have investigated the phenotypic differences between these classes. We compared 20 non-deletion to 20 age-matched deletion patients and found significant phenotypic differences between the two groups. The more severe phenotype in the deletion group may suggest a contiguous gene syndrome.

Keywords: Angelman syndrome; deletion 15q11–q13; uniparental disomy; imprinting mutation; UBE3A mutation; GABRB3 gene

Introduction

Angelman syndrome (AS) is a severe neurodevelopmental disorder with a heterogeneous genetic aetiology. After its initial description in 1965[1] the clinical AS phenotype has been well characterised.[2] Common manifestations include severely delayed motor development, mental retardation, speech impairment, gait ataxia, epilepsy with abnormal EEG, as well as physical anomalies such as microcephaly, characteristic facial phenotype, hypopigmentation and scoliosis.

AS results from the lack of contribution of normally active maternally-inherited genes on chromosome 15q11–q13. Interstitial deletions of chromosome 15(q11–q13) account for 70% of cases (class I). In the 30% of cases showing no deletion, about 3–5% result from paternal uniparental disomy (UPD) (class II), and about 5% are due to imprinting mutations (class III). In

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the remaining 20% of patients, including numerous familial cases, no molecular abnormality was detectable until the recent finding of UBE3A mutations (class IV).[3,4]

The variability of AS phenotype has been described in few reports, but most of these ante-date the recognition of UPD, imprinting mutations, and abnormalities of UBE3A gene.[5–8] Phenotypic comparison between deletion and non-deletion AS patients has been reported in only two studies.[9,10] There were no significant differences except for hypopigmentation, found only in deletion cases. A milder phenotype in paternal UPD cases has been suggested by different authors.[11–14] On the other hand, Prasad *et al*[15] reported one UPD case without a 'substantially different phenotype from the average severity in deletion case'. Two recent reports of nine and five imprinting mutation cases respectively [16,17] described no significant differences in the phenotypic spectrum of AS except for more frequent occurrence of microcephaly and hypopigmentation in deletion cases.

In 89 AS patients diagnosed in our Department of Medical Genetics, we found basically the same frequency of main AS manifestations as reported in the literature.[2,18] Apart from the pigmentary abnormalities associated with deletion cases,[9] non-deletion patients nevertheless seemed to have a milder phenotype than deletion ones. To confirm this putative genotype–phenotype correlation, we compared the clinical manifestations of a group of 20 non-deletion AS patients consisting of UPD, imprinting mutations, and UBE3A mutations to those of an age-matched control group of 20 deletion cases.

Subjects and Methods

The 40 patients were part of a survey of 89 AS patients diagnosed between 1989 and 1996, and evaluated by the same clinical geneticist and neuropaediatrician. Since the phenotypic expression of AS is age-dependent,[8] each non-deletion patient was compared to an age-matched patient with a deletion. The 20 AS patients of both groups were further divided into three categories: ten children (4–13 years), three adolescents (15–17 years), and seven adults (18–36 years). The following physical and neurological parameters were systematically evaluated: height, weight and head circumference, ability to walk and age of onset, epilepsy with age of onset and type of seizures, tremulous movements, language performance, communication abilities. Microcephaly was defined as a head circumference smaller than the mean value by at least two standard deviations.[19] Skin, hair, and iris pigmentation was evaluated by comparison with that of the parents and normal sibs.

A combination of molecular and cytogenetic analyses was used to classify patients according to molecular groups as previously defined.[20] Procedures for DNA extraction, DNA methylation test with probes PW71B (D15S63) and SNRPN 5'-end, microsatellite studies, cytogenetic analysis, and FISH followed standard protocols.[3,21–31] In patients with biparental inheritance of chromosome 15 and normal methylation status, recent molecular screening for UBE3A mutations was initiated by SSCP analysis; it will be described elsewhere (Malzac *et al*[32]). The two groups of AS patients are as follows:

The deletion group included seven females and 13 males. All cases were sporadic. In two individuals, the deletion arose as the result of an unbalanced *de novo* translocation: 45,XY,der(1) t(1;15) (qter–q13),–15 and 45,XY, der(15) t(15;22) (q12–p11), –22. Three of the patients including those with unbalanced translocation have been previously reported.[33,34] The origin of the deleted chromosome was maternal in probands. Not all patients were tested or were informative for each probe, but there was sufficient information to establish that the deletion spanned the entire region commonly deleted in AS.[35]

The non-deletion group included five females and 15 males. There were nine sporadic cases and 11 familial cases from five unrelated families: two sets of siblings (two females with consanguineous parents and one family with a male and a female sibling), and three larger families (two brothers and one female first cousin; one male, two first cousins and a maternal uncle, and one family with seven affected cases in three generations). In the latter two families, two cases are currently being evaluated. The molecular findings in this group are as follows:

1 Three sporadic patients with paternal UPD (class II).

2 Three sporadic cases with an abnormal methylation pattern with PW71B and/or SNRPN and biparental inheritance of chromosome 15 (class III).

3 Three sporadic cases and all the familial cases had biparental inheritance of chromosome 15 and normal methylation. Recent screening for

UBE3A mutations detected three frameshift mutations in the sporadic cases (exon 8, exon 10, exon 15), five mutations in the familial cases with four frameshift mutations (exon 9, exon 10, exon 12, exon 16), and one amino-acid insertion mutation (exon 15) (class IV).

For both groups of patients, the frequency (quantitative parameter) and age of onset (qualitative parameter) of the symptoms were studied. To assess whether differences in distribution of the frequencies of a particular manifestation were significant, we used Fisher's exact test. If the data concerning a clinical parameter was unknown, the patient and the age-matched patient were excluded from the calculation (values given Table 1). The null hypothesis that the frequencies of respective symptoms in deletion and non-deletion patients are different was rejected at the 5% level ($P > 0.05$). Qualitative parameters were studied by analysis of variance (ANOVA). Because both groups were small, significance was confirmed by the Mann-Whitney parametric test.

Results

The distribution of each of the major manifestations of AS for the two groups is presented in Table 1.

Microcephaly

In deletion patients, a microcephaly was present by 2 years in 18 of 20 patients (90%) (Figure 1).

In non-deletion patients, a microcephaly was present in seven of 20 cases (35%) (Figure 1). No patients from either group had a head circumference above the mean value.

The P value is significant for microcephaly ($P < 0.001$).

Ability to Walk and Age at Onset

In the deletion patients, 15 of 20 patients (75%) could walk independently at the last review. Mean age of walking was 5.2 years and varied between 2.5 to 10 years (Figure 2). Of the five patients (25%) who could not walk independently, two were aged 5, and the others were 6, 22 and 23 years old. In the adult group two patients showed loss of ambulation and became wheelchair-bound.

All non-deletion patients could walk independently. The age at which walking began varied between 19 months and five years of age; mean value was 30 months (Figure 2). None presented with walking difficulties as they grew older.

The P value for ability and age of walking between the two groups was significant, 0.047 and 0.001 respectively.

Epilepsy

All patients with a deletion had epilepsy which started before 3 years of age in 18/20 cases. Age of onset varied between 6 months and 5 years (Figure 3), with a mean at 20 months. The main ictal patterns were atypical absence seizures, myoclonic-atonic seizures, and tonic-clonic seizures. Infantile spasms were observed in two cases. Myoclonic status with decreased alertness and loss of smile, lasting several days or weeks, was reported in four patients. In all cases, EEG showed the

Table 1 Distribution of each major manifestation between deletion and non deletion cases

Groups of patients	*Deletion*			*Non deletion*			
Clinical evaluation	*Mean age*	*number*	*%*	*Mean age*	*number*	*%*	P *value*
Age at diagnosis (years)	11.4			12.8			NS
before 3 years		8/20	40		4/20	20	
4 to < 18 years		6/20	30		9/20	45	
≥ 18 years		6/20	30		7/20	35	
Microcephaly		18/20	90		7/20	35	<0.001
Delay in growth HC		19/20	95		15/20	75	NS
Ability to walk		15/20	75		20/20	100	0.047
Age of onset (years)	5.2			2.6			<0.001
Seizures		20/20	100		14/20	70	0.02
Age of onset (years)	1.8			5.4			0.004
Cortical myoclonus		17/19			18/20		NS
Growth retardation (-2SD)		10/20	50		2	10	0.013
Adult group		5/7	71.5		2/7	28.6	
Obesity (≥ + 2SD)		3/20	15		10/20	50	0.013
Overweight (≥ + 1SD)		9/20	45		15/20	75	NS
Hypopigmentation		15/20	75		0/20	0	< 0.001

typical pattern for AS:[35,36] high voltage, atypical slow spike waves with a maximum over the frontal or occipital regions and sometimes diffuse. All patients were treated with valproic acid and/or benzodiazepines. In the child group, seizures continued but with very low activity in seven cases; three of them became seizure-free 2–3 years after treatment initiation. The three remaining child cases were more severe. In the adolescent and adult group, epilepsy never improved in four cases, and the frequency and duration of epileptic seizures became worse in adult age. In four cases, there was recurrence during adulthood. In only two adult cases did epilepsy and EEG pattern show improvement with age.

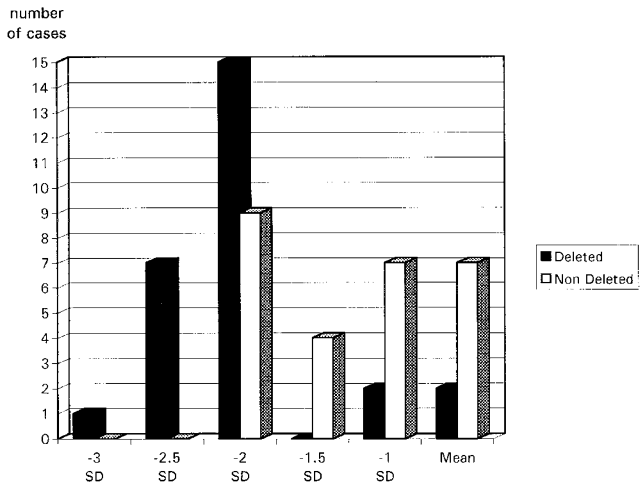

Figure 1 *Distribution of head circumference (HC) between 20 AS patients with deletion and 20 AS patients without deletion.*

Tremor, recently related to fast-bursting cortical myoclonus, occurred periodically in the majority of the deletion patients (17/19 cases).[37] We observed that this phenomenon appeared more pronounced in adolescents and adults, increasing when triggered by stress or strain, and that it could lead to typical myoclonic seizures and/or myoclonus status.

In non-deletion cases, epilepsy was present in 14 of 20 cases (70%). Age at onset ranged from 6 months to 20 years with a mean age at 5.4 years (Figure 3). The most frequent ictal patterns were atypical absences and myoclonic seizures. In three cases (one adolescent and two adults), generalised clonic seizures were observed. Seizures remained rare in all patients and cessation of treatment was possible in some cases. The seven

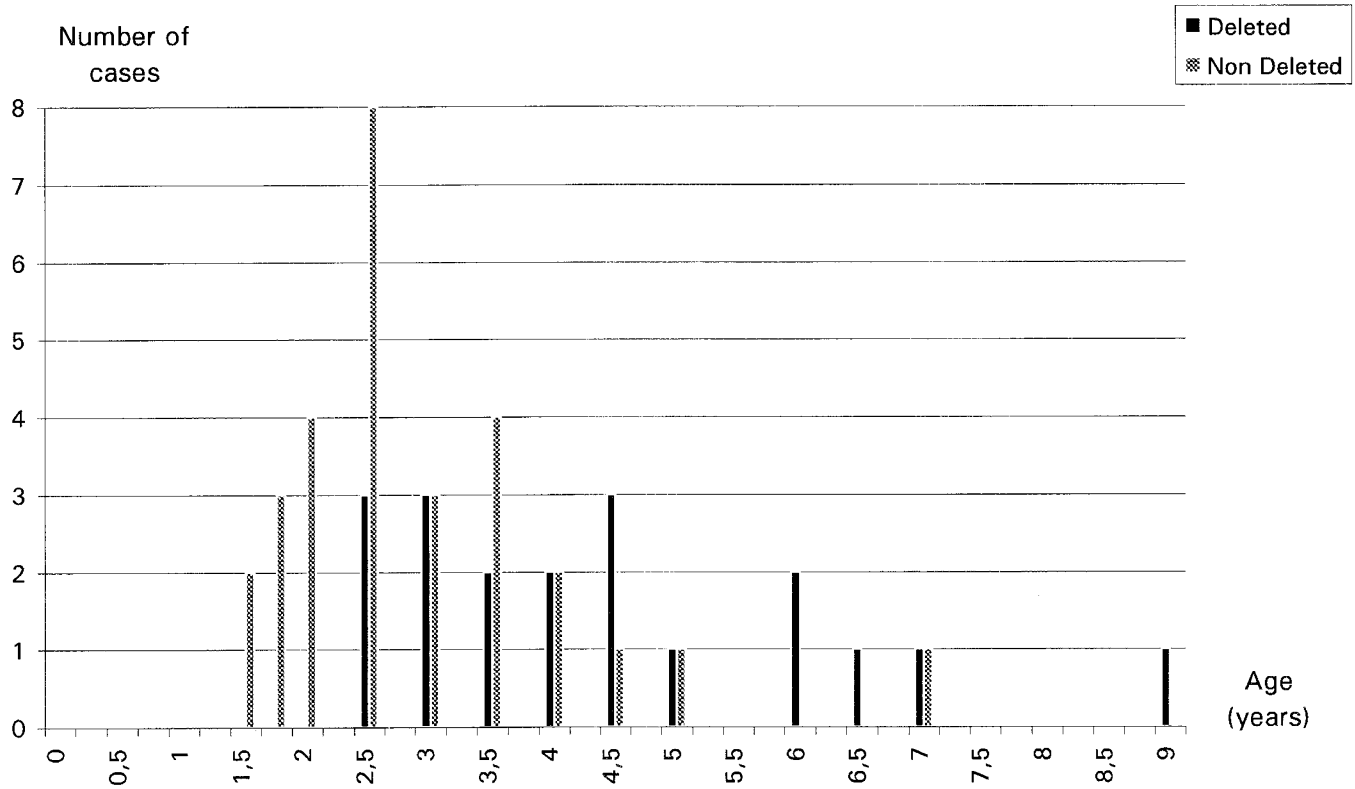

Figure 2 *Distribution of age of onset of walking between 15 AS patients with deletion and 20 AS patients without deletion (5 AS deleted patients did not walk at the last review).*

seizure-free patients were aged from 4 to 30 years. However, EEG patterns were abnormal in all cases whatever their age.

Tremor also occurred in the majority (18/20), either with or without seizures but was often more pronounced with increasing age. It was correlated with onset or recurrence of typical myoclonus seizures and/or myoclonus status in adolescents or adulthood (three cases).

P value of frequency of epilepsy and age of onset between the two groups was significant (0.02 and 0.004, respectively).

Language and Communication

None of the deleted patients had developed any speech, except 'papa, mama', or rare speech-like syllables. Severe oral motor dyspraxia was a consistent finding. Patients never acquired any ability to execute familiar oral acts, like kissing, on command. Performing a single order was usually possible, if the child was well motivated. Imitation skills remained poor. Patients were not able to imitate expressive motor patterns except for occasional waving or clapping. In non-verbal communication, most children had the ability to express their basic needs and food preferences. They usually used repeated touching to get attention and guided adults by the hand to what they wanted, but neither pointing nor communication through gestures was established. The vast majority of teenagers and adults were totally dependent in daily life, requiring assistance with feeding, toilet, and dressing.

Most of the non-deletion patients were able to acquire a few words (usually four to ten words). Four subjects could say 20 'words' or more. In fact, patients only utter one or two syllables of each word, with very defective articulation. Oral praxies were deficient, but kissing became possible for most of them. Patients were able to designate parts of the body or pictures (animals), to execute complex orders, and to imitate motor patterns and some simple gestures. Their non-verbal communication was particularly efficient, with ability to use pointing and, above all, to elaborate a private gestural code quite clear to other family members. The vast majority of teenagers and adults were able to dress and feed themselves without assistance.

Height and Weight

In deletion patients, height was retarded by –2 SD in 10 of 20 cases (50%). In the adult cases, there was a definitive small height in five of seven cases (Figure 4). Excessive weight (≥1 SD relative to height) was present in 9/20 cases (45%) (4/10 children, 2/3 adolescents, and 3/7 adults). Obesity (≥2 SD above normal value) was present in three of 20 cases (15%) (in one child, one adolescent, and one adult).

In non-deletion patients, growth was retarded by –2 SD in only two adults (10%) (Figure 4). Growth rate was within normal value in all the remaining cases (12 cases at mean value, five at +1 SD). Above average weight was present in 15/20 cases (75%) (7/10 children, 2/3 adolescents, and 6/7 adults). Obesity was present in 10/20 (five children, one adolescent, and four adults). The *P* values for growth retardation and obesity between the two groups were significant at 0.013 and 0.013, respectively, whilst that for overweight was not.

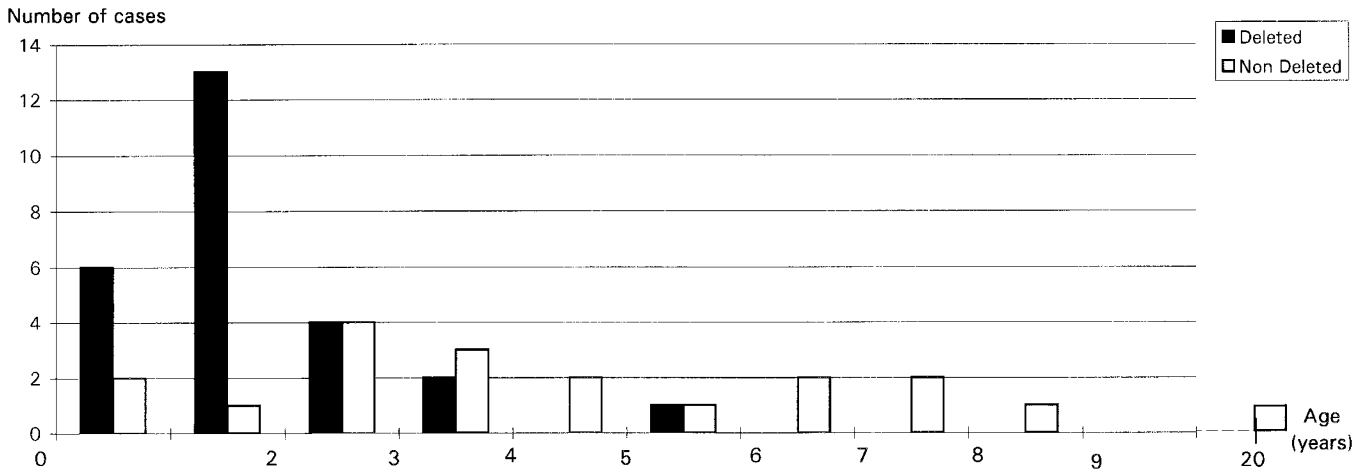

Figure 3 *Distribution of age of onset of seizures between 20 AS patients with deletion and 20 AS patients without deletion (six AS non-deletion patients did not present seizures at the last review.)*

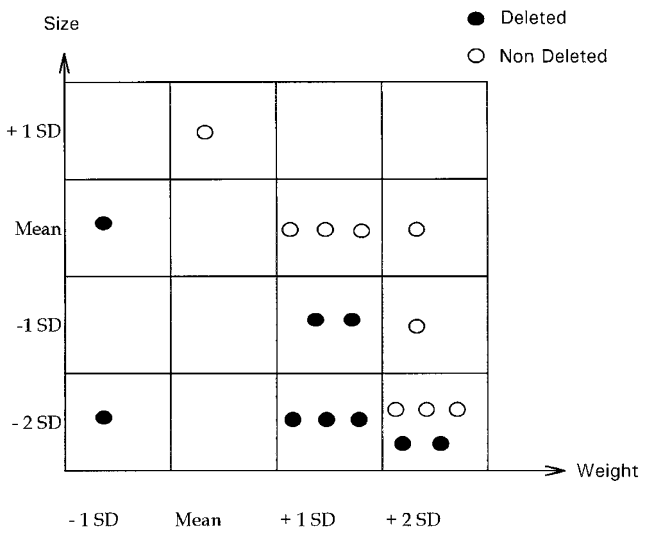

Figure 4 *Distribution of height and weight between seven AS adults patients with deletion and seven AS adults patients without deletion.*

Pigmentation

In deletion patients, hypopigmentation was a feature in 15/20 cases (75%). In one adult, it was present during childhood but was less evident with age. In one child of Italian origin, pigmentation appeared to be normal. The other three patients were adults and no data was available for early age. Among non-deletion patients, none was hypopigmented. The *P* value for hypopigmentation between the two groups was significant ($P < 0.001$).

Discussion

We have compared the clinical phenotype of 20 molecularly well defined non-deletion AS patients with that of 20 deletion patients. To the best of our knowledge, this is the first age-matched comparative clinical study in AS to include in addition the study of both qualitative and quantitative parameters for each major clinical manifestation of the syndrome.

Table 1 summarises the comparison between the two groups. Our results statistically demonstrate that a less severe phenotype is associated with non-deletion AS with respect to both physical anomalies and neurological manifestations.

Deletion and non-deletion AS patients have been compared in only two reports,[9,10] but in these papers the non-deletion groups are not molecularly well defined. More recent studies compared molecularly well defined small groups of AS patients – those with UPD[14] or imprinting mutations[16,17] with those with classical deletion. In all these reports, only the frequency of each major manifestation of AS was evaluated. Clinical details have been reported in two large surveys of deletion patients (37 from Japan[9] and 27 from Australia[39]). To date, a total number of 39 molecularly well characterised non-deletion patients have been clinically described including 16 UPD[11–15,40] 14 imprinting mutations,[16,17] and nine with UBE3A mutations.[41–43] We excluded reports of familial AS with incomplete molecular analysis.

The present study demonstrates a clear difference in the degree of microcephaly between the two groups (90% in deletion and 35% in non-deletion). In the deletion group, microcephaly is more frequent than in previous reports,[9,39] although an exact comparison is difficult to make due to differences in patient ages and measurement parameters used. In the total number of 39 non-deletion patients, microcephaly is observed in 16 cases (41%).[11–17,40,41,43]

In addition, facial dysmorphism appeared milder in 13/20 non-deletion cases in comparison with the typical facial appearance observed in 19/20 of the deletion patients. This difference was more obvious in the adult group (Figure 5) but should be confirmed by objective facial measurements.

A significant difference in body height was observed between the two groups. Growth retardation was associated only with deletion and UBE3A mutations. All patients with imprinting mutations or UPD were within the mean range for height. A significant difference was also noted in the frequency of obesity between the two groups. Increase in weight often began in late childhood, and was correlated with hyperphagia. Parents described a behavioural phenotype (food seeking and stealing) similar to the one associated with Prader-Willi syndrome (PWS). This observation points to an overlap between AS and PWS as previously reported in two AS patients.[44] Recently, Cattanach *et al*[45] described an AS mouse model with partial paternal disomy for the syntenic region. Interestingly, this mouse model exhibits obesity. Few data are available on growth parameters in AS[18,39,46] and further clinical studies on large AS series are needed to confirm these results.

Study of neurodevelopmental parameters in both patient groups demonstrated a less severe phenotype in the non-deletion group. Ability to walk independently and age at onset were significantly different between the two groups. Two studies also reported earlier onset

of walking in non-deletion patients.[17,14] Age of onset and ability to walk appear dependent on the degree of expression of two parameters: ataxia and seizures. Moderate to very mild ataxia is consistently found in the non-deletion group as well as a less severe epilepsy phenotype. Concerning epilepsy, we have seen distinctive patterns between the two groups. In the deletion cases, epilepsy always began early, prior to 3 years of age. It was often severe, especially during childhood. Even when seizures were less frequent, treatment needed to be maintained. In non-deletion cases, epilepsy was not a consistent manifestation and was less severe. The age of onset varied greatly from infancy to adulthood. The frequency of epilepsy previously reported for deletion patients was 100% and 96%.[9,38] In non-deletion patients, epilepsy is less frequent; it was reported in 19/38 cases (50%).[11–17,40,41,43]

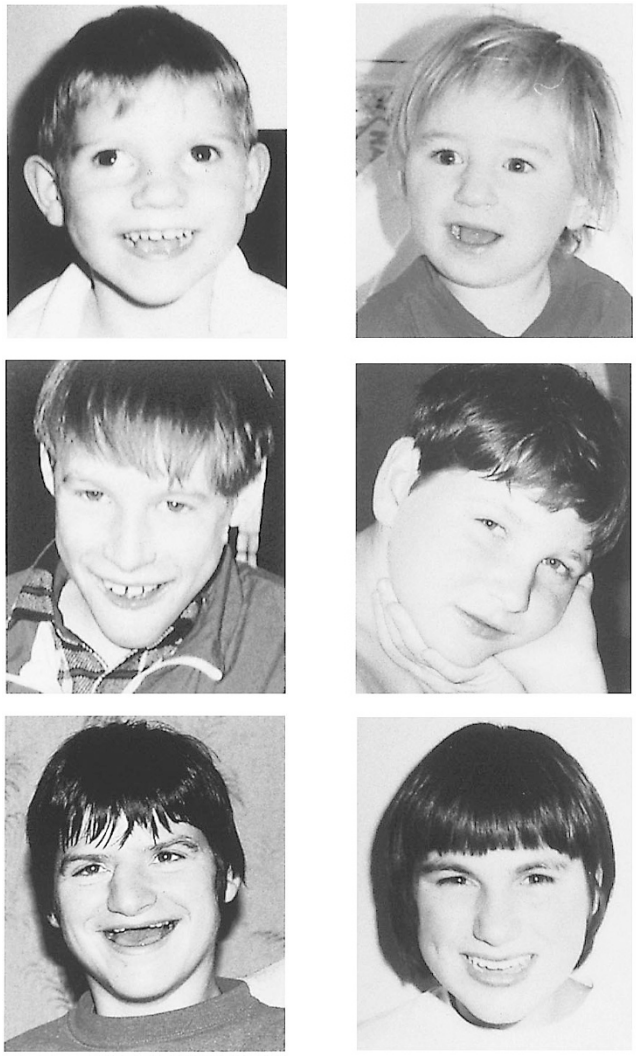

Figure 5 *Comparison of facial dysmorphism between patient with deletion and patient without deletion (left: deletion patient; right: non-deletion patient).*

Severe mental retardation and a marked lack of expressive speech are obvious and permanent features of AS in late infancy. However, clinical evaluation enabled us to find significant variations in the level of cognitive abilities and communication skills. Such differences had been noticed previously in a study of 11 AS cases with only the familial patients able to imitate and learn a few words.[47]

In addition, we observed a different distribution of both physical and neurological parameters between the three molecular classes of the non-deletion group (Table 2). Microcephaly is less frequent both in imprinting mutations (3/17; 17.7%) and UPD (6/19; 31.6%) compared with UBE3A mutations (14/22; 63.6%). Similarly, epilepsy was less frequent both in UPD (8/17; 47.1%) and imprinting mutations (11/17; 64.7%) compared with UBE3A mutations (17/23; 74%). Our patients with UBE3A mutations presented a more significant variability both in age of onset and frequency of crisis compared to those with UPD and imprinting mutations, and only some patients with UBE3A mutation showed growth retardation.

This study raises the question of whether mutation or deletion of only the imprinted *UBE3A* gene can explain the more frequent microcephaly and the severe epilepsy phenotype found in patients with common large deletion. The deleted region includes a cluster of $GABA_A$ receptor subunit genes (*GABRB3*, *GABRA5*, and *GABRG3*). These genes are involved in inhibiting synaptic transmission and are highly expressed in embryonic brain.[48] Initially, *GABRB3* was proposed as an AS candidate gene,[49] but it was then excluded because a subtle microdeletion not encompassing the

Table 2 Frequency of epilepsy and microcephaly among the three molecular classes of our non-deletion group and previous reports

	UPD			*Imprinting mutation*			*UBE3A mutation*		
	Present study	*Other**	*Total %*	*Present study*	*Other*[a]	*Total %*	*Present study*	*Other**	*Total %*
Microcephaly	1/3	5/16	31.6	0/3	3/14	17.7	6/14	8/8	63.6
Epilepsy	2/3	6/14	47	2/3	9/14	64.7	9/14	8/9	74

[a]references 11–17, 40, 41, 43.

gene cluster was found in one AS patient[50] and because of its biallelic expression in mouse brain.[51] Recently, targeted disruption of *GABRB3* has been performed and homozygous mutant mice exhibit epilepsy, EEG abnormalities, and a phenotypic behaviour similar to AS.[52] Hence, the role of these genes in the AS phenotype cannot be definitively excluded but remains unclear.

In conclusion, deletion AS cases correspond well to the initial clinical description of Angelman.[1] Cases without deletion appear to have a milder expression of the main neurological manifestations and physical anomalies. These findings are particularly important for clinical diagnosis, because a milder phenotype can be associated with molecular classes having a potential high risk for recurrence. A clinical severity scale from more to less severe can be summarised as follows: deletion cases > UBE3A mutation cases > imprinting mutation and/or UPD cases. This type of phenotype–genotype correlation study represents a first step towards further understanding of both the molecular basis of AS and the role of imprinting in this syndrome.

Acknowledgements

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