

## ELECTRONIC LETTER

## Severe phenotype in Angelman syndrome resulting from paternal isochromosome 15

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Angelman syndrome (AS) is a neurogenetic disorder with an occurrence of approximately 1/20 000 live births.<sup>1</sup> Characteristic features include severe mental retardation, absence of speech, seizures, abnormal EEG, hyperactivity, happy disposition with unmotivated laughter, ataxia of gait, and physical anomalies such as microbrachycephaly, macrostomia, protruding tongue, and widely spaced teeth.<sup>2</sup> The clinical diagnosis of AS is usually not suspected during the first years of life because the early features are non-specific and the diagnosis is usually made between the ages of 2 and 16. In adults, the clinical diagnosis is often difficult because some characteristics, such as hyperactive behaviour, bursts of laughter, seizures, and EEG pattern tend to improve with age.<sup>3</sup> As a result, the frequency of AS is probably underestimated.

AS is caused by the functional absence of the maternal copy of 15q11-q13. This region is subject to genomic imprinting, whereby gene expression is dependent on the parent of origin.<sup>4</sup> In about 70% of cases, it is caused by de novo maternal deletions in the 15q11-q13 region, in approximately 2-3% by paternal uniparental disomy (UPD) of chromosome 15, and in 3-5% by imprinting mutations. The remaining AS patients (20%) have biparental inheritance. Some of these cases result from intragenic mutations in the *UBE3A* gene.<sup>5-6</sup> About 20% of AS families in the imprinting mutation and biparental inheritance groups have more than one affected relative.<sup>2,7-8</sup>

Prader-Willi syndrome (PWS) is located in the same region. This syndrome is caused by the functional absence of the paternal copy of 15q11-q13. PWS is a phenotypically distinct disorder from AS and is characterised by infantile hypotonia, hypogonadism, mild to moderate mental retardation, hyperphagia (leading to obesity), short stature, small hands and feet, and characteristic facial appearance.<sup>9</sup> Some of these characteristics, such as hyperphagia and obesity, have been observed in some AS patients with UPD and imprinting mutations.<sup>10-15</sup>

Phenotype-genotype correlation in AS is complex, but some investigators suggest that the phenotype in UPD is milder than in deletions.<sup>12-14,16-18</sup> They report better physical growth, fewer or no seizures, less ataxia, less severe epilepsy, earlier onset of walking, and higher cognitive abilities and communication skills. However, other authors have not observed differences between deletion and UPD.<sup>19,20</sup>

Genetically, deletion involves the loss of a fragment of a chromosome, whereas uniparental disomy is the abnormal inheritance of two copies of a chromosome from only one parent. In AS patients with UPD, both homologous chromosomes 15 are inherited from the father; this situation has a well documented, abnormal phenotype, contributed by presumed imprinted loci. For its part, UPD can be considered to be of two types, heterodisomy, the inheritance of two different copies of a gene or chromosome from one parent, and isodisomy, the inheritance of two identical copies of a gene or chromosome from one parent. In AS, isodisomy is the more frequent situation. It can result from meiotic or mitotic duplication of one parental chromosome and may involve isochromosomes,



**Figure 1** Phenotype of patient 2.

whereas heterodisomy may involve Robertsonian translocation. Only four cases with de novo balanced t(15q15q) karyotypes have been described. In these cases, DNA polymorphism analysis has indicated paternal UPD, suggesting that the structural rearrangement in these cases was a 15q isochromosome, not a Robertsonian translocation.<sup>11,14,17,21</sup> An isochromosome has genetically identical arms and may form through centromere misdivision<sup>22,23</sup> or a U type exchange in the proximal short arm region or centromere.<sup>24-26</sup>

## CASE REPORTS

### Patient 1

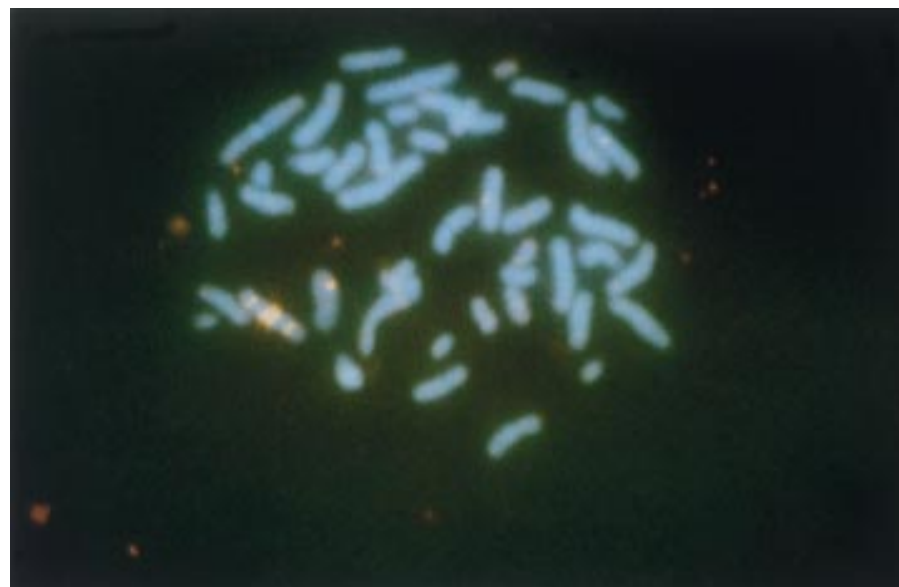
This male child was born on 21.10.88, after a controlled pregnancy and birth by caesarian section, because of a previous caesarian. Birth weight was 3560 g and birth length was 50 cm. Apgar scores were 9/10. His mother was 33 years of age and his father 35. The parents reported that he cried a great deal while still on milk. A change of character was noted after the first DPT vaccination dose at 7 months, with the child becoming very irritable. On the second DPT vaccination dose, at 8 months, he was admitted to hospital with a high fever and showed West's syndrome. At 12 months, he suffered a crisis with slow spike waves on the EEG, coinciding with fever and drop attacks. Since then he has been treated with clonazepam and valproic acid. In general, the crises have been well controlled, the last EEG being normal. He has axial hypotonia of the limbs and extremities. He can maintain himself erect, though somewhat awkwardly, for some seconds with anterior and lateral support. Physical examination at the age of 9 years showed a height of 130 cm (50th centile), weight of 28 kg (50th centile), head circumference of 50.5 cm (3rd centile), brachycephaly, occipital groove, macrostomia, and widely

**Abbreviations:** AS, Angelman syndrome; PWS, Prader-Willi syndrome; UPD uniparental disomy

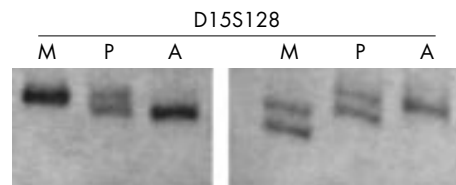


**Figure 2** Partial metaphase from proband 2 showing the Robertsonian translocation  $t(15q15q)$  with G banding (arrow).

spaced teeth. Neither hypopigmentation nor ocular alterations were observed. Neurological examination showed severe mental retardation and serious ataxia which impeded independent walking, although he has been able to move around with help since the age of 7 years. At rest he shows motor hyperactivity. He has normal muscle tone, with abductor and ischiotibial hypertonia and is also hyper-reflexive. No asymmetry was noted. There is an absence of language and of communication by gestures. His behavioural phenotype corresponds to AS, with a happy disposition, laughter attacks, easily excitable personality, hand flapping, disrupted sleep (which has improved with treatment using melatonin), fascination with water, masticating movements, and hypersensitivity to heat, all of which were reported at the age of 9 months. At the age of 6 years, a cranial MRI scan showed



**Figure 3** FISH results of proband 2. Two signals, corresponding to D15S10 and PML probes, can be observed on each arm of the translocated chromosome 15.



**Figure 4** Molecular studies. Cases 1 and 2 inherited a D15S128 allele from their father. Paternal UPD is evident in both cases by lack of the maternal allele. M: maternal, P: paternal, A: patient.

hypoplasia of the corpus callosum and the vermis, with an increase of the cisterna magna.

#### Patient 2

This male child was born on 11.4.91 after a controlled pregnancy and term birth. Birth weight was 3100 g and birth length was 50 cm. Apgar scores were 9/10. His mother was 24 years old and his father was 26. At the age of 3 years, he was admitted for a convulsive episode, which had begun at 7 months, in the form of distal polymyoclonia and almost continual episodes of myoclonic absences and astatic myoclonic crisis. The EEG showed a globally destructured graph with slow wave complexes. He responded to treatment with valproic acid and the convulsive episodes and EEG anomalies disappeared. The following characteristics stood out in the physical phenotype (fig 1): obesity (75th centile) from the age of 1 year, short stature (3rd centile), small hands and feet, marked hypotonia with a major lack of postural control, hypogonadism, somnolence, round facies, and hypopigmentation, all suggesting a possible diagnosis of PWS. However, the typical behavioural phenotype of AS was verified. Some neurological and clinical symptoms of AS, such as severe mental retardation, profound speech impairment, jerky movements, microcephaly, smiling demeanour, and hyperactive behaviour, were present at 4 years of age. Ataxia was moderate and at this age he still did not walk. The EEG clearly showed a poor, non-convulsive status. No alterations of the wake-sleep rhythm were seen. A cranial CT scan showed cortico-subcortical atrophy. There was persistence of obesity (28.6 kg, >97th centile), height of 100.3 cm (10th centile), hypogonadism, and hypotonia at this age. The child died at home at the age of 7 years.

#### METHODS AND RESULTS

Cytogenetic analyses in these patients were performed on peripheral blood samples, set up in a 72 hour culture of high

**Table 1** Clinical characteristics in AS patients with UPD

Clinical characteristics	Isochromosome (n=6) 45,XY,t(15q;15q)						Isodisomy (n=10) 46,XX or 46,XY									
	Patient 1	Patient 2	Freeman <i>et al</i> <sup>11</sup>	Tonket <i>et al</i> <sup>17</sup>	Ramsden <i>et al</i> <sup>21*</sup>	Fridman <i>et al</i> <sup>14</sup>	Botanni <i>et al</i> <sup>12</sup>				Gillessen-Kaesbach <i>et al</i> <sup>16</sup>	Beuten <i>et al</i> <sup>19</sup>	Prasad & Wagstaff <sup>20</sup>	Smith <i>et al</i> <sup>13</sup>		Smith <i>et al</i> <sup>27</sup>
<i>Consistent and frequent (more than 80%)</i>																
Age (y)	9	5	3	2.5	9	9	7.5	10	5		10	7	8	11	4.5	30
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Speech <3 words	0	6			8	3	5	5	0		0	10	15	8	3	6
Gestures/commun	-	+		+	+	+	+	+	+	+	-	+	+	+	+	+
Ataxia	+	+/-			+	+	+/-	+/-	+	+	+	+/-	+/-	+	+/-	+/-
Frequent laughter	+	+	+		+	+	+	+	-	+	+	+	+	+	+	+
Hand flapping	+	+		+	+		-	+								
Hyperactivity	+	+	+	+	+		+	+		+	+	+	+	+		
Microcephaly	+	+	-	+	+	-	-	+	+			-	-	+	-	-
Abnormal EEG	+	+	+		+		+	+	+	+		+		+		+
Seizures	+	+	-	-	+	+	+	+	+	+	+	-	-	+	-	+
Age of seizure onset (y)	0.6	0.6			7	6.8	4.5	3	5		5			1.5		
Age of walking onset (y)	7	5	3.5	2	2.5	2.7	2.5	2.3	2.5		6	2	2	2.5	2	3
<i>Associated (20-80%)</i>																
Flat occiput	+	+/-			-		-	+								
Protruding tongue	-	-	-		+		-	+	-							
Feeding problems	-	-	-		-		-	+								
Prognathism	-	-	+		+		-	-		+	+				+	+
Wide mouth	+	-	-		+	+	-	+	+	+	+	+	+	+	+	+
Frequent drooling	+	+			+	+	+	+								
Hypopigmented	-	+	+		-		-	-	-							
Hyperphagia	-	+			-	+	+	+								+
Weight (centile)	50	97	95	6	ND	98	97	90			90	50	10	97	97	97
Height (centile)	50	10	90	50	ND	98	97	25	25		90	75	25	3	90	90
Phenotype	Not milder	Not milder	Milder	Milder	Milder	Milder	Milder	Milder	Milder	Milder	Not milder	Not milder	Milder	Milder	Milder	Milder

n: number of cases. ND: not determined.

\*Revised by us.

**Table 2** Clinical characteristics of AS patients with UPD

Clinical characteristics	UPD					
	Isochromosome (n=6)		Isodisomy (n=10)		Total (n=16)	
	No	%	No	%	No	%
<i>Consistent and frequent (more than 80%)</i>						
Developmental delay	6/6	100	9/9	100	15/15	100
Speech <3 words	1/4	25	2/9	22.2	3/13	23
Gestures/commun	4/5	80	7/8	87.5	11/13	84
Ataxia	3/4	75	4/10	40	7/14	50
Frequent laughter	5/5	100	8/9	88.89	13/14	92
Hand flapping	4/4	100	1/2	50	5/6	83.3
Hyperactivity	5/5	100	7/7	100	12/12	100
Microcephaly	4/6	66.6	3/8	37.5	7/14	57.1
Abnormal EEG	4/4	100	7/7	100	11/11	100
Seizures	4/6	66.67	7/10	70	11/16	68
Age of seizure onset (y)		Mean=3.76		Mean=3.8		Mean=3.78
Range		0.58–7		1.5–4.5		0.58–7
Age of walking onset (y)		Mean=3.78		Mean=2.75		Mean=3.2
Range		2–7		2–6		2–7
<i>Associated (20–80%)</i>						
Flat occiput	1/3	33.33	1/4	25	2/7	28
Protruding tongue	1/4	25	1/3	33.33	2/7	28
Feeding problems	0/4	0	1/2	50	1/6	16
Prognathism	2/4	50	4/6	66.6	6/10	60
Wide mouth	3/5	60	8/9	88.89	11/14	78
Frequent drooling	4/4	100	2/2	100	6/6	100
Hypopigmented	2/4	50	0/3	0	2/7	28
Hyperphagia	2/3	66.67	3/3	100	5/6	83
Weight (>50th centile)	3/5	60	6/8	75	9/13	69.2
Height (>50th centile)	2/5	40	5/9	55.5	7/14	50

resolution G banding, and showed a de novo, non-mosaic, apparently balanced Robertsonian translocation or isochromosome in all 20 cells examined, with a 45,XY,t(15q15q) karyotype (fig 2). Their parents' karyotype was normal.

Fluorescence in situ hybridisation (FISH) was carried out in patient 2 using *SNRPN* and D15S10 probes from VYSIS. These probes contain two control markers for identification of the 15p arm (D15Z1) and the distal region 15q22 (*PML*), respectively. This patient showed absence of the D15Z1 sequence in the translocated chromosome, while the normal hybridisation pattern of the *SNRPN* or D15S10 and *PML* probes (two signals on each arm of the translocated chromosome) were observed, indicating the absence of a deletion in the PWS/AS critical region (fig 3).

To determine the parental origin of the t(15q15q), five highly polymorphic microsatellite markers from chromosome 15 were used, at loci D15S11, *GABRB3*, D15S128, D15S210, and D15S122. DNA was extracted from peripheral blood leucocytes from the probands and their parents, using standard techniques. After electrophoresis of the PCR products on an 8% non-denaturing polyacrylamide gel, the DNA fragments were visualised by silver staining. The results at five different chromosome 15 loci showed that patients 1 and 2 had inherited both chromosomes 15 from their father, not from their mother, indicating a paternal uniparental disomy. The fathers were heterozygous, and the fact that the patients had inherited only one of the two paternal alleles for locus D15S128 was informative, showing that the t(15q15q) was an isochromosome, with two copies of the same allele (fig 4).

We compared the clinical histories of these two new cases of isochromosome 15 with four published cases of isochromosomes and 10 published cases of isodisomy (table 1). Other reported cases of UPD were mainly reciprocal translocations yielding a heterodisomy. We included them in our study to preserve the homogeneity of this group and because the clinical data were not reported. The clinical manifestations of these 16 UPDs are summarised in table 2. We observed that our

patients showed a more severe phenotype than the rest of the cases, with the exception of those cases described by Beuten *et al*<sup>19</sup> and Prasad and Wagstaff.<sup>20</sup> This greater severity was characterised by poorer verbal and non-verbal communicative capacity in patient 1, who does not use any words and whose capacity to communicate by gestures is limited. Patient 2, however, had some communicative capacity. Seizures manifested themselves prematurely in both patients, at 8 and 7 months, respectively, but not in the other patients, where the median age of onset of seizures was 3.8 years. Our two patients began to walk late, at 7 and 5 years of age, respectively. This was also the case in the patient of Prasad and Wagstaff,<sup>20</sup> who did not begin to walk until the age of 6 years. Nevertheless, seizures manifested themselves late, at 5 years of age, and, as in our patient, his communication was limited. All these features taken together mean that, as in our two cases, this patient's condition could be considered severe. Beuten *et al*<sup>19</sup> described a patient with isodisomy as severe, though the clinical description was not particularly thorough. A comparison of the clinical parameters of isochromosome and isodisomy groups of patients using Fisher's test showed no significant differences.

## DISCUSSION

UPD is infrequent (2%) in AS. As a result, information on these patients is scarce. Approximately 30 cases, counting both isodisomies and heterodisomies, have been reported, isodisomies being more frequent. Of the isodisomies, only four originated from isochromosome 15.<sup>11 14 17 21</sup> This paper reports two new cases of isochromosome 15. These two patients and those described by Beuten *et al*<sup>19</sup> and Prasad and Wagstaff<sup>20</sup> represent 25% of the UPDs analysed (4/16). In our view, this phenotype is as severe as that reported in deletion cases; it is characterised by poorer verbal and non-verbal communicative capacity, early manifestation of seizures, and a late age of onset of walking (6 years). Because of this severity, these cases do not support the hypothesis formulated by Bottani *et al*,<sup>12</sup>

### Key points

- Angelman syndrome (AS) resulting from uniparental disomy (UPD) is relatively infrequent. Most AS patients have a maternal deletion of 15q11-q13 and show severe developmental delay, ataxia, absence of speech, and a happy disposition.
- Several authors have reported that the phenotype observed in UPD may be milder than in deleted cases, because patients with AS resulting from UPD show better physical growth, fewer or no seizures, less ataxia, less severe epilepsy, earlier onset of walking, and higher cognitive abilities and communication skills.
- In this paper, we report two patients with AS resulting from UPD caused by isochromosome 15, identified by cytogenetic banding, fluorescence in situ hybridisation (FISH), and DNA analysis, who showed most of the major clinical criteria of Angelman syndrome and a phenotype that does not differ greatly from that found in deleted cases. Our two patients had poor verbal and non-verbal communicative capacity, early onset seizures (8 and 7 months), and began to walk late at 7 and 5 years of age, respectively.
- In the light of these observations and other reports, we suggest that a severe phenotype may be found in around 25% of cases of AS resulting from paternal UPD. A review of the clinical phenotypes of 14 AS patients with UPD is presented for comparison with our two cases.

according to which patients with AS resulting from UPD present a milder phenotype than deleted AS patients. Other authors have subscribed to this hypothesis.<sup>13 14 16-18 27</sup> However, we should stress that a small proportion of UPD patients show unusual features such as hyperphagia and obesity, seen in our patient 2 and in the patients of Bottani *et al.*,<sup>12</sup> Smith *et al.*,<sup>27</sup> and Fridman *et al.*<sup>14</sup> These characteristics are not typical of AS and may easily be confused with the features of PWS.

We investigated the possibility that the severity of the phenotype may have been the result of a reorganisation in the isochromosome. To this end, we compared the isochromosomes with the isodisomies, but no significant differences were observed, perhaps because of the small number of patients involved. Besides, among the group of patients with isodisomy, there are two cases<sup>19 20</sup> who also show a severe phenotype like our patients with isochromosome, so there may be other causes. Finding a genetic explanation for these observations is not easy. Various genes are involved in the clinical manifestation of AS and not all of them are known. Some patients with clinical diagnoses of AS do not have molecular alterations in 15q11-q13. Furthermore, this region is regulated by the mechanism of imprinting, which further complicates our understanding of how these genes are expressed in the development of the different tissues.

In fact, the observation of a less severe phenotype in some UPD patients with imprinting mutations supports the idea that other genes besides the imprinted genes are involved in UPD patients with a severe phenotype. The situation of UPD and imprinting mutation is similar in the sense that they principally affect imprinted genes. At this time, it is not clear which non-imprinted genes might be involved.

Smith *et al.*<sup>27</sup> proposed that growth regulating genes may be responsible for the less severe evolution of UPD patients. They suggested that UPD patients have two active copies of paternal genes which promote normal growth, whereas growth in deleted AS patients is delayed. Thus, a possible explanation for the severe UPD phenotype might be deficiency of both paternal copies. Another possible cause could be a low level trisomy

15 mosaicism. Olander *et al.*<sup>28</sup> reported a severe phenotype in a boy with mosaic PWS resulting from maternal UPD with mosaic trisomy 15. In addition to the typical PWS phenotype, this patient also had a cardiac defect. The severe phenotype in our UPD AS patients could be the result of residual trisomy 15; however, no other anomalies that distinguish our subjects from deletion patients were found.

We conclude that the majority of patients with UPD show a milder phenotype, but there are some cases (~25%) in which the phenotype differs little from that found in deleted cases. In addition, AS patients with a severe phenotype present unusual traits that are characteristic of PWS, such as hyperphagia, obesity, and hypotonia. Therefore, given the clinical heterogeneity of AS, it is vital to take into account clinical traits that are not included in the major criteria, together with confirmation of molecular studies, in order to determine the possible phenotypic differences according to aetiology.

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