ELECTRONIC LETTER

A patient with a supernumerary marker chromosome (15), Angelman syndrome, and uniparental disomy resulting from paternal meiosis II non-disjunction

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The chromosome 15 region q11-q13 is imprinted and contains a number of genes that are expressed only from the paternally or the maternally inherited chromosome. This region is also prone to structural rearrangements including interstitial duplications¹ and triplications,² inversions,³ translocations,⁴ deletions,⁵ and the formation of supernumerary marker chromosomes (SMCs).^{6,7} These rearrangements are associated with a wide range of abnormal phenotypes depending upon both the nature of the rearrangement and on the parental origin. For example, Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are distinct neurobehavioural disorders that are both caused by a deletion of 15q11-q13.⁵ A deletion on the maternally inherited chromosome 15 gives rise to AS while a paternally inherited deletion causes PWS. These conditions can also be caused by uniparental disomy (UPD) of chromosome 15: maternal UPD cases will be functionally nullisomic for those genes expressed only from a paternally inherited chromosome and gives rise to PWS, while paternal UPD causes AS.

Additional copies of the Prader-Willi/Angelman syndrome critical region (PWACR) have also been reported and can occur as familial cases or arise de novo. Unlike deletions, additional copies of the PWACR appear to be associated with an abnormal phenotype only when inherited maternally.^{8,9} These additional copies can occur as interstitial duplications/triplications or as SMC(15). SMC(15) is the most common marker chromosome observed in man, accounting for 50% of all cases.¹⁰ There are two basic types: large SMC(15) extend over most or all of the q11-q13 region, including the PWACR, and are associated with abnormal phenotypes^{6,11}; small SMC(15) do not contain the PWACR and are not generally associated with an abnormal phenotype,^{11,12} although they have occasionally been shown to occur in association with other disease causing abnormalities such as 15q11-q13 deletions¹³ and UPD(15).¹⁴ In all large and small de novo cases where the origin has been determined, the SMC(15) has been shown to be derived maternally.

We report a boy with an abnormal phenotype and a de novo SMC(15). Molecular and molecular cytogenetic analysis showed that the SMC did not include the PWACR. This investigation fortuitously showed that the boy had inherited both chromosome 15 homologues from his father, indicating paternal UPD consistent with a diagnosis of AS. Interestingly, the UPD in this case is likely to have arisen because of paternal non-disjunction at meiosis II followed by trisomy rescue and is the first reported case of its kind.

CASE REPORT

The patient is the first child of unrelated parents with no notable previous family history. He has one younger brother who is normal. Both parents were aged 27 at the time of his birth. He was delivered normally at term, presented no neonatal problems, and was healthy with no dysmorphic features. He sat at 6 months, but from the age of 18 months there

was evidence of significant delays in the development of both language and motor skills: he had no speech, had poor coordination, and exhibited hand clapping and flapping reminiscent of AS. When assessed at the age of 15 years, he had moderate to severe learning disability. Verbal communication was limited to the use of about half a dozen words but he was able to communicate in a number of other ways such as pointing and gesturing. He was only a little unsteady on walking. He did not have seizures and was normally pigmented. During routine cytogenetic analysis in 1996 a SMC was found, to which his abnormal phenotype was attributed at the time. Further studies were initiated later as part of a project investigating the effects of additional copies of the PWACR.

METHODS

Karyotypes were determined by analysis of G banded metaphase chromosomes harvested from peripheral blood lymphocytes. FISH studies based on standard methods¹⁵ were carried out using the probes cos27 (D15S13) and pTRA-25.

Molecular analysis using microsatellite markers across chromosome 15 was performed as described previously.¹ Dosage PCRs were carried out using primers from chromosome 15 (UBE3A and D15S63) and control primers from chromosome 5. Reaction conditions were the same as for the microsatellite markers except that the magnesium concentration was increased to 2.5 mmol/l. A denaturation step of 94°C for five minutes was followed by 20 cycles of 94°C, 55°C, and 72°C, each of 45 seconds. A final extension of 72°C for five minutes was followed by a 60°C hold of one hour.

Bisulphite analysis was carried out using a technique modified from Zeschnigk *et al.*¹⁶ Fluorescent primers specific for either the maternal or paternal methylation pattern were used and the number of PCR cycles reduced to 20 to keep within the linear amplification range.

RESULTS

Cytogenetic analysis identified the presence of a SMC in the proband. Parental karyotypes were normal. FISH with probes for the chromosome 15 centromeric region (pTRA-25) and the PWACR (D15S13) showed that the SMC originated from chromosome 15 but did not contain the PWACR (fig 1). Molecular analysis with microsatellite repeat markers in the PWACR confirmed that the SMC(15) was negative for this region. The SMC(15) did not include the microsatellite markers D15S541 and D15S542, which are seen in approximately 50% of small SMC(15).¹² Small SMC(15) are not generally

Abbreviations: SMC, supernumerary marker chromosome; AS, Angelman syndrome; PWS, Prader-Willi syndrome; UPD, uniparental disomy; PWACR, Prader-Willi/Angelman syndrome critical region

Figure 1 FISH image with cosmid probe cos27 (D15S13) (red) and plasmid probe pTRA-25 (green) on metaphase chromosomes from the proband. Cos27 signals are seen on both chromosome 15 homologues, but not on the SMC(15), indicating the absence of the PWACR on the marker chromosome.

associated with abnormal phenotypes and so this SMC is likely to be coincidental to the abnormalities in this patient.

The molecular results showed that only paternal alleles were present for chromosome 15 (table 1). Dosage PCR combined with FISH excluded a deletion and indicated that the lack of maternal alleles was the result of paternal UPD. Bisulphite analysis of the family also confirmed the absence of a maternal specific methylation pattern for the *SNRPN* gene in the proband. For the molecular markers proximal to D15S118, heterozygosity in the father is reduced to homozygosity in the proband (table 1). However, for markers distal to D15S118 heterozygosity is retained in the proband indicating that the chromosomes are heterodisomic and excluding a postzygotic mitotic origin for the UPD. The reduction of centromeric markers and the transition to non-reduction of more distal markers strongly indicates that the UPD arose by a meiosis II non-disjunction error in the father during spermatogenesis. A meiosis I error cannot be excluded, but is extremely unlikely given that the interval between the centromere and the most proximal marker (D15S541) is 0 cM.¹⁷

DISCUSSION

We have identified a patient with AS caused by paternal heterodisomy of chromosome 15 and who also carries a de novo SMC(15). To the best of our knowledge, this is the first such patient to be described. Only two similar patients with AS, paternal UPD, and a small SMC(15) have been described previously. Robinson *et al*¹⁴ described a boy with paternal isodisomy of chromosome 15 and a small de novo SMC(15). In this case, the most likely explanation for the UPD was a postzygotic non-disjunction event. Lebar *et al*¹⁸ described a

girl with a small SMC(15) and paternal UPD but with no molecular details. AS cases as a result of UPD caused by paternal meiosis II errors have been reported,^{19,20} but not in the presence of a SMC(15).

Small SMC(15) have been shown to occur in association with del(15) and UPD(15) and their frequency in PWS cases is greatly increased compared with the normal population (1 in 40 as opposed to 1 in 1000).²¹ In all these cases, the abnormal phenotype was attributable to either the deletion or the UPD and the presence of the small SMC(15) was not thought to contribute to the phenotype. These observations of small SMCs in PWS and AS cases have led to the suggestion that one type of error predisposes to the other¹³ and also highlights the need for detailed phenotype/genotype correlations in patients with SMC(15).

It has been reported that AS patients with UPD exhibit milder phenotypes than deletion cases^{22,23} and that the incidence of AS may have been underestimated owing to milder phenotypes occurring outside the normal AS spectrum.²⁴ A milder phenotype in UPD compared with deletion cases suggests a contiguous gene syndrome, with the deletion of non-imprinted genes in this region also contributing to cause a more severe phenotype.²⁵ The severity of our patient's phenotype is thus of interest. He does not have seizures and nor does he suffer from the severe gait disturbances normally seen in AS patients. Almost 90% of AS patients are unable to speak,²³ whereas our patient is able to use a small number of words and communicates in a number of other ways. Normal pigmentation is seen in our patient, while many AS patients are hypopigmented, presumably because of haploinsufficiency for the non-imprinted pigmentation *P* gene. The patient's facial features and behaviour,

Table 1 Microsatellite analysis on 15q11-q13 of proband and parents. The markers are shown in order from the centromere towards the telomere. Those markers highlighted in bold indicate paternal UPD. The sex averaged genetic distances from the centromere are taken from Robinson and Lalonde.¹⁷ Allele status in the proband is shown in the last column

	Genetic distances (cM)	Microsatellite marker	Proband	Mother	Father	Homozygosity
Cen	0	D15S541	2 2	2 3	1 2	R
	0	D15S542	1 1	1 2	1 1	NI
PWACR		D15S1035	1 1	2 3	1 1	NI
	0.5	D15S543	1 1	1 2	1 1	NI
	1.7	D15S11	3 3	1 2	2 3	R
	2.6	D15S128	2 2	1 3	1 2	R
		D15S1506	1 1	2 2	1 2	R
	6	GABRB3	3 3	1 4	2 3	R
		D15S822	2 2	1 3	2 3	R
		D15S1002	1 1	1 3	1 2	R
	10.7	D15S219	1 1	1 1	1 1	NI
		D15S1019	2 2	1 2	1 2	R
		D15S1048	3 3	1 3	2 3	R
	15.1	D15S165	3 3	1 2	1 3	R
		D15S118	1 1	2 4	1 3	R
		D15S114	2 4	1 3	2 4	NR
		D15S117	1 2	3 4	1 2	NR
Tel		D15S127	1 2	2 2	1 2	NR
		D15S207	2 2	1 3	2 2	NI

R, reduced; NR, not reduced; NI, non-informative.

including jerky movements and frequent laughter, are reminiscent of AS, but at the milder end of the AS spectrum. This case provides further evidence that UPD AS patients have less severe phenotypes than deletion cases.

AS caused by paternal UPD is relatively rare, accounting for only 2-5% of cases. The majority of maternal UPD(15) cases occur via maternal meiosis I segregation errors.^{26,27} The majority of the small number of AS paternal UPD cases observed are isodisomic and are thought to be the result of postzygotic mitotic events (mechanism 3 or 4).²⁸ The UPD in our patient has arisen by a paternal meiosis II error and is not typical of most chromosome 15 UPD cases.

The occurrence of two relatively rare chromosomal abnormalities in this patient is unlikely to be coincidental. Two possible mechanisms can explain the simultaneous presence of the SMC(15) with UPD that has arisen meiotically. (1) In a trisomic zygote, the single maternal chromosome 15 homologue underwent a rearrangement to form a SMC, thereby reducing the chromosome 15 complement to two. (2) Both the UPD and the SMC(15) were generated by related events in the father, in which case the absence of a maternal contribution may be explained by a nullisomic gamete or by removal of the maternal chromosome 15 by trisomy rescue. However, the second mechanism is unlikely because no SMC was identified in the father's peripheral blood and because all de novo SMC(15) studied molecularly have been shown to be maternally derived. This strongly suggests that paternal non-disjunction occurred independently during spermatogenesis and subsequent formation of a SMC(15) by the maternal chromosome 15 occurred to rescue the trisomic zygote.

This case is, as far as we know, the first of its kind to be reported. While small SMC do not themselves cause abnormal phenotypes, they are frequently associated with other disease causing abnormalities including UPD(15), deletions, and duplications. This highlights the need to screen for other abnormalities in carriers of small SMC(15) with abnormal phenotypes.

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