Uniparental disomy explains the occurrence of the Angelman or Prader-Willi syndrome in patients with an additional small inv dup(15) chromosome

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Abstract

A patient with Angelman syndrome and a 46,XY/47,XY,+inv dup(15)(pter→q11:q11→pter) karyotype and a patient with Prader-Willi syndrome and a 46,XY/47,XY,+inv dup(15)(pter→q12:q12→pter) karyotype were investigated with molecular markers along chromosome 15. Paternal uniparental isodisomy was found for all informative markers in the first case which indicates that this, rather than the presence of the extra chromosome, is the cause of the Angelman syndrome phenotype. Similarly, the PWS patient showed maternal uniparental disomy with absence of PWS region material on the inv dup(15) chromosome. If (1) marker chromosomes are an occasional by product of 'rescuing' a trisomic fertilisation, or (2) if duplication of the normal homologue in a zygote which has inherited a marker in place of the normal corresponding chromosome 'rescues' an aneuploid fertilisation, or (3) if the presence or formation of a marker chromosome increases the probability of non-disjunction, then uniparental disomy might be found occasionally in other subjects with de novo marker chromosomes.

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Angelman syndrome is associated with a maternal deletion of 15q11-q13 in more than 50% of cases¹ or, much more rarely, paternal uniparental disomy (UPD) of chromosome 15 is found.²⁻⁴ Prader-Willi syndrome patients show a paternally derived deletion of the same region in about 75% of cases with maternal UPD(15) in virtually all remaining typical patients.⁵⁻⁷ This pattern of inheritance implies that there are at least two imprinted genes in this region, one active only on the maternally derived chromosome 15 and another active only on the paternally derived one.

The presence of a small extra marker chromosome derived from chromosome 15 is an occasional finding in Prader-Willi syndrome patients,⁸⁻¹⁴ but has not previously been reported in Angelman syndrome. These marker chromosomes are generally small and include only short arm material and occasionally some heterochromatic material from the q arm¹⁵ and are therefore unlikely to include the Prader-Willi syndrome critical region in

15q12, as defined by minimum regions of deletion overlap in PWS patients.

It has been hypothesised previously by us that the karyotype of such patients probably originated as a trisomy for chromosome 15 with loss of one chromosome through marker formation occurring by an early postzygotic breakage/duplication event.¹⁶ If the marker formed from the single paternal chromosome, in a maternally derived trisomy 15, this would result in maternal UPD(15) for the normal pair. This would be somewhat similar to the proposed mechanism to explain the observation that fetuses showing trisomy 15 in placental biopsy and a normal diploid karyotype at birth may occasionally show maternal UPD(15) and the associated PWS phenotype.^{17,18} This hypothesis would predict that if the same mechanism occurred in a paternally derived trisomy 15 it would be possible to lose the single maternal chromosome, occasionally with gain of a maternally derived marker, resulting in paternal UPD for the normal pair of chromosomes 15 and the Angelman syndrome phenotype. An alternative hypothesis, also resulting in UPD(15), is that the presence or formation of a marker chromosome during meiosis may increase the probability of non-disjunction and therefore UPD. In this case the marker could originate from either parent since there is either a loss or gain of a chromosome 15 from both parents.

To investigate this possibility, we examined the inheritance of markers on the normal chromosomes 15 in an AS and a PWS patient both with a 46,XY/47,XY,+inv dup(15) karyotype.

Methods and results

CASE 1

The proband, a 4 year old male, was the only child born, after an uneventful pregnancy, to a 30 year old mother and 40 year old father. Birth weight was 2990 g, birth length was 50 cm, and head circumference was 34 cm. At 6 months, a slow down in psychomotor acquisition was noted. He sat at 9 months and walked at 24 months. An abnormal EEG was found at 2 years and antiepileptic medication was prescribed. A CT scan showed asymmetry of the lateral ventricles with the right ventricle more dilated than the left. Gait was unsteady, language was delayed, and trunk ataxia and tremors during fine movements were noted.

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Figure 1 QFQ stained metaphase from case 1. The supernumerary chromosome is indicated by larger arrow, smaller arrows point to the two normal chromosomes 15.

The external genitalia were normal. At 4 years the proband's height, weight, and head circumference were within the normal range. A clinical diagnosis of Angelman syndrome was made although no hypopigmentation was present.

CASE 2

At 12 years, this male patient showed all the classical signs of PWS including marked obesity resulting from hyperphagia, cryptorchidism, hypogenitalism, narrow forehead, and mental retardation. Neonatal hypotonia and feeding problems in infancy were present. Height was between the 10th and 25th centiles.

CYTOGENETICS

Cytogenetic analysis of a blood culture from case 1 showed the karyotype 46,XY/47,XY,+ inv dup(15)(pter→q11:q11→pter) in a ratio of 40:60. Dystamycin-A DAPI staining showed two bright DAPI positive ends, indicating origin from chromosome 15. CBG staining showed that there were two distinct centromeres. Differential staining properties of the two ends of the marker were also seen with QFQ staining and indicate its origin from two different chromosomes 15 (fig 1). The inv dup(15) appears to contain very little of the q

arm, and is presumably composed entirely of heterochromatic material. The chromosomes of the parents were normal.

Cytogenetic analysis of a blood culture from case 2 showed the karyotype 46,XY/47,XY,+ inv dup(15) in a ratio of 30:70. The inv dup(15) chromosome in this case is slightly larger and could be interpreted as inv dup(15)(pter→q12:q12→pter) or inv dup(15)(pter→q11:q12→pter) (fig 2). This chromosome, however, appears smaller than those we have analysed previously which contained one or two copies of the PWS region.¹⁶ The parental chromosomes were normal.

Material was no longer available in either case to perform in situ hybridisation of PWS/AS region probes and thereby to confirm directly the content of the inv dup(15) chromosomes.

MOLECULAR ANALYSIS

Hybridisation of probes to DNA from the probands and parents were performed as described previously.⁶ Probes used included pIR39 (D15S18), p34 (D15S9), p3-21 (D15S10), pIR4-3 (D15S11), pIR10-1 (D15S12), and p189-1 (D15S13),¹⁹⁻²¹ all mapping within the PWS deletion region of 15q11-q13. In addition the VNTR probes CMW-1 (D15S24),²² located in 15q13, and ms620 (D15S86, mapping near the 15q telomere)²³ were also used. Microsatellite loci for D15S11,²⁴ GABRB3,²⁵ GABRA5,²⁶ D15S97, CYP19, D15S108, ACTC, D15S87, and IPM15M9 (Genome data base) were detected by PCR amplification using ³²P labelled cytosine and visualised on a 6% polyacrylamide denaturing gel.

Case 1 showed no maternal inheritance for the D15S13 RFLP and the GABRB3 microsatellite loci, both mapping within the PWS/AS deletion region (fig 3A), and for the IPM15M9 microsatellite and D15S86 VNTR locus (fig 3B), mapping to distal chromosome 15 (table). Reduction of paternal heterozygosity to homozygosity in patient 1 was observed for all informative loci on chromosome 15: D15S18, GABRB3, GABRB5, D15S24, IPM15M9, and D15S86.

The father was not available for study in case 2. However, the patient was identical to the mother at all loci, whereas the brother showed inheritance of non-maternal (paternal) alleles (table). Dosage analysis was performed with a Molecular Dynamics 'Personal Densitometer' and showed copy numbers in the patient of: IR39: 3.3; p34: 2.3; 28β3-H3: 2.1,1.9 (two different blots); p189-1: 1.9; and 3-21:2.1 (fig 4). Thus, there was no evidence for a duplication of any loci except for the most proximal locus detected by IR39 (D15S18), which is also often not included in the deletion in many PWS and AS deletion cases.^{1,27,28} Because only 70% of cells contained the extra marker chromosome, an average dosage of 3.3 copies for p39 indicates that two copies of this locus were present on the inv dup(15) chromosome.

Methylation studies using hybridisation of

Figure 2 Partial G banded karyotype from case 2 showing the D group chromosomes and marker.

Figure 3 Molecular results supporting paternal isodisomy of chromosome 15 in case 1. (A) PCR amplification was uninformative for the D15S11 microsatellite, but shows inheritance of a single paternal allele and no maternal allele at GABRB3. (B) Hybridisation of probe ms-620, mapping on distal 15q, shows only paternal inheritance.

PW71 to *HindIII*/*HpaII* digests were used to confirm the parental origin of the PWS region material.²⁹ PW71 (locus D15S63) detects a parent of origin specific methylation pattern which has proven reliable in the diagnosis of deletions and UPD(15) involved in PWS and AS syndrome without requiring parental DNA. Our own unpublished results also indicate that the parent of origin methylation pattern is conserved in all rearrangements involving 15q11–13, including in eight patients with a maternally derived inv dup(15) chromosome that includes the PWS/AS region, in two patients with direct duplications of the PWS/AS region, in two cases with atypical PWS deletions, and in one ring(15) chromosome case.

In case 1, only the unmethylated, paternal band was present, confirming that there was no maternal inheritance of PWS region genes. In case 2, only the methylated maternal band was present, indicating that there was no paternal inheritance of PWS region genes (fig 5). As this patient is heterozygous in this PWS region

and shows a normal diploid copy number, maternal uniparental heterodisomy of the two normal chromosome 15 homologues is the most likely explanation for the molecular results.

Discussion

Paternal isodisomy for the entire long arm of chromosome 15 was found in case 1 and is the explanation for the expression of the AS phenotype. The extra chromosome appears to contain mostly or only heterochromatic p arm material, which should have no phenotypic effect. The parental origin of the marker chromosome could not be proven, although it seems likely to be maternal, since that is the chromosome 15 which is lacking. Maternal UPD(15) was found in the PWS patient, case 2. Although the father was not available for study, this diagnosis can be confirmed based on the observation of no paternal transmission determined using parent of origin methylation differences detectable by probe PW71, no evidence of a duplication (or a deletion) by dosage of probes in the PWS/AS critical region, and markers consistent with maternal UPD(15) throughout the chromosome 15.

The presence of homozygosity for markers along chromosome 15 in case 1 indicates that the second paternal chromosome 15 may have arisen as a postzygotic non-disjunction event rather than a meiotic one. In other words, the original karyotype in the zygote is likely to have been 46,XY,–15,+inv dup(15), with rescue of the zygote by duplication of a single paternal chromosome. Instability of the marker could explain the mosaicism. Alternatively, the zygote may have originated as a trisomy with the maternal chromosome being lost through a breakage/duplication event. In this case, the paternal non-disjunction event must have been the result of either a meiotic I non-disjunction with recombination occurring only between D15S18 and the centromere or a meiotic II non-disjunction without meiosis I recombination. Lastly, the duplication of the father's chromosome and the formation of the inv dup(15) chromosome may have been related events.

The results of case 2 are consistent with origin from a trisomic conception with loss of the paternal chromosome 15 through a breakage/duplication event resulting in an inv dup(15) chromosome.¹⁶ It is also possible that the inv dup(15) chromosome was present before fertilisation in the gamete from either parent. Parental origin of the extra chromosome could not be determined. However, the second maternal chromosome presumably arose by a meiosis I non-disjunction event, as markers nearest to the centromere showed inheritance of both maternal alleles.

Although the majority of patients with small chromosomes derived from 15 which contain only heterochromatic material should be normal, such cases appear to be at an increased risk for maternal or paternal UPD(15) which would result in the PWS or AS phenotype. Thus, when such a finding occurs at prenatal

Results of DNA analysis. Probes are shown in map order from centromere to telomere. Probes D15S9 to D15S12 are deleted in most PWS and AS deletion patients. Allele designations are arbitrary and are different for cases 1 and 2.

Locus	Probe/enzyme	Location	Case 1			Case 2		
			Proband	Mother	Father	Proband	Mother	Brother
D15S18	39/BglII	15q11.2-12	22	22	12	2222	22	22
D15S9	34/ScaI	15q11.2-12	11	11	11	11	11	12
D15S11	PCR	15q11.2-12	aa	aa	aa	ab	ab	ab
D15S13	189-1/TaqI	15q11.2-12	22	11	22	—	—	—
D15S10	3-21/TaqI	15q11.2-12	11	11	11	—	—	—
GABRB3	PCR	15q11.2-12	aa	bc	ad	ab	ab	bc
D15S97	PCR	15q11.2-12	—	—	—	ab	ab	bc
GABRA5	PCR	15q11.2-12	aa	—	ab	ab	ab	bc
D15S12	ScaI	15q11.2-12	22	22	22	—	—	—
D15S24	CMW1/EcoRI	15q13	aa	aa	ab	—	—	—
ACTC	PCR	15q13-15	aa	—	aa	—	—	—
CYP19	PCR	15q21	aa	ac	ab	—	—	—
D15S99	PCR	15q22-25	aa	—	ab	—	—	—
IPM15M9	PCR	15q25	aa	cd	ab	—	—	—
D15S86	ms620/EcoRI	15q26	cc	ab	bc	—	—	—
D15S87	PCR	15q26	—	—	—	ab	ab	bc

— = not typed.

Figure 4 Dosage analysis of p34 and pIR39 in case 2 (lane 1) relative to a control, H2-42, from chromosome 13 shows a normal two copy dosage for p34 but higher dosage (estimated average copy number of 3.3) for pIR39. Lane 4 is an atypical AS patient without a deletion, lane 6 is an AS deletion patient, and lane 7 is a patient with an inv dup 15 including a duplication of the PWS/AS region.

Figure 5 Parent of origin specific methylation detected by hybridisation of PW71 to HindIII/HpaII digests. Lane 1 is the PWS patient (case 2) which shows only a maternally derived methylated 6.0 kb band.

diagnosis, exclusion of UPD(15) should be considered. UPD should also be considered as an explanation for an abnormal phenotype in patients with small marker chromosomes derived from other chromosomes. The abnormal phenotype could be the result of either duplication of genes near the centromere, or imprinting effects, or homozygosity for rare recessive mutations on the two cytogenetically normal homologues. For the majority of human chromosomes, it is still unknown what effects imprinting may have, and a search for UPD among subjects with de novo marker chromosomes seems a possible approach to determine this.

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