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# EXCLUSION OF UNIPARENTAL INHERITANCE OF CHROMOSOME 15 IN A FETUS WITH A FAMILIAL DICENTRIC (Y;15) TRANSLOCATION

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## SUMMARY

We present a prenatal case with a 45,X,dic(Y;15) (q11.23;p11.1) karyotype and describe the inheritance pattern of the chromosome 15s. Chromosome 15 has an imprinted region and inheritance of both chromosome 15 from one parent results in either Angelman syndrome (AS) (paternal inheritance) or Prader–Willi syndrome (PWS) (maternal inheritance). Parental chromosome studies revealed that the father carried the same dicentric (Y;15) translocation. Since familial chromosome rearrangements can result in aberrant chromosomal segregation during meiosis, we wanted to exclude paternal uniparental inheritance of chromosome 15. By using DNA microsatellite markers at several 15q11q13 loci, we determined that the fetus had inherited his normal non-translocated chromosome 15 from his mother. © 1998 John Wiley & Sons, Ltd.

KEY WORDS: dicentric (Y;15) chromosome; uniparental disomy; microsatellite; 15q11q13; imprinting

## INTRODUCTION

Genomic imprinting refers to the differential expression of genes dependent on whether they are inherited from the father or the mother. Chromosome 15q11q13 is a genetically imprinted region that has two phenotypically distinct mental retardation syndromes associated with it. Absence or disruption of maternally derived chromosome region 15q11q13 results in Angelman syndrome (AS), while loss or disruption of its paternally derived counterpart results in Prader–Willi syndrome (PWS) (Butler and Palmer, 1983; Buiting *et al.*, 1995; Donlon *et al.*, 1986; Knoll *et al.*, 1989; Magenis *et al.*, 1989; Nicholls *et al.*, 1989a,b; Williams *et al.*, 1990; Mascari *et al.*, 1992; Malcolm *et al.*, 1991). AS is characterized by infantile hypotonia, unfounded bouts of laughter, severe mental retardation, ataxic movements, absent speech, disturbed sleep patterns, and seizures (Williams *et al.*, 1995). PWS children have hypotonia and failure to thrive during infancy, hyperphagia leading to obesity in early childhood, mild to moderate mental retardation, temperature sensitivity, short stature, and small hands and small feet (Holm *et al.*, 1993).

Several aetiologies causing disruption of 15q11q13 exist for both syndromes. They include microdeletions, uniparental inheritance, and biparental inheritance with or without imprinting centre mutations. The specific aetiology that will be discussed in this paper is uniparental inheritance/disomy (UPD), which results from inheriting both chromosome 15s from one parent. Maternal UPD results in PWS (Nicholls *et al.*,

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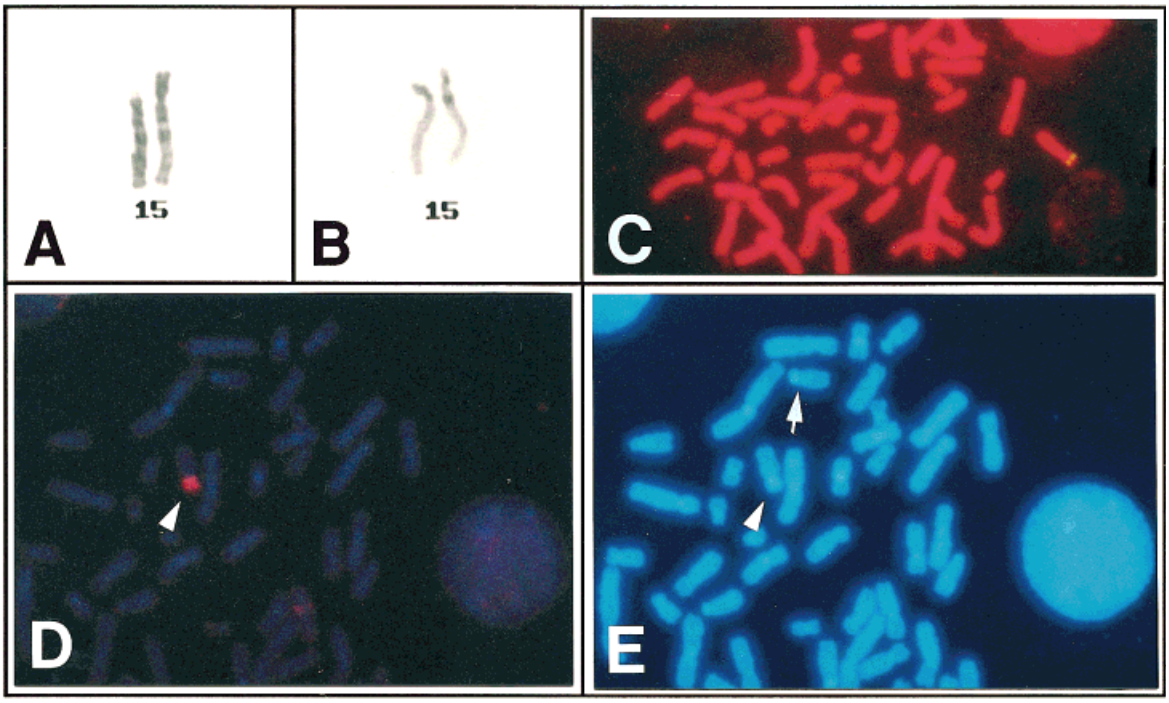


Fig. 1—(A) GTG-banded chromosome 15s with abnormal chromosome 15 on the right. (B) C-banded chromosome 15s with two C-bands evident on the abnormal chromosome 15 on the right. (C) DYZ3 hybridization on abnormal chromosome 15. (D) WCP-Y hybridization on abnormal chromosome 15 (arrow-head) with (E) corresponding DAPI image for chromosome identification. Normal chromosome 15 is indicated (arrow)

1989a; Mascari *et al.*, 1992) and paternal UPD results in AS (Malcolm *et al.*, 1991). UPD results from non-disjunction and is caused either by the reduction of a trisomic zygote to a disomic one or by the correction of a monosomic zygote to a disomic one (Spence *et al.*, 1988). There is increased likelihood of aberrant segregation leading to non-disjunction when a familial translocation or other chromosomal rearrangement is present (Toth-Fejel *et al.*, 1996). We present a case in which we used DNA markers to determine the parental origin of the normal chromosome 15 in a fetus with a paternally inherited chromosome 15 translocation.

## MATERIALS AND METHODS

### *Case report*

A chorionic villus biopsy was obtained at 10 weeks on the second pregnancy of a 37-year-old gravida 2, para 0 female for chromosome analysis. Both direct and cultured preparations for chromosome analysis were set up using standard procedures (Simoni *et al.*, 1983; Holmes *et al.*, 1988).

### *Cytogenetics and fluorescence* in situ *hybridization (FISH)*

Chromosomes were GTG-banded (Seabright, 1972) and analysis of cells from both the direct and the cultured preparations showed 45,X,dic(Y;15)(q11.23;p11.1). A partial karyotype is shown in Fig. 1A. The origin of the chromatin on the short arm of chromosome 15 (15p) was determined during chromosome analysis on a previous pregnancy referred for advanced maternal age. In that pregnancy, the karyotype was 47,XX,15p+,+21. At that time, parental chromosomes were studied and it was determined that the phenotypically normal father had a 45,X,15p+ karyotype. QFQ (Caspersson *et al.*, 1970) and CBG banding (Sumner, 1972) were performed to determine the nature of the additional chromatin on 15p. QFQ banding showed that the 15p+ was negative for

Table I—Results of microsatellite analysis

| Locus (primer name) | | Mother | Father | Fetus* | Reference† |
|---|---|---|---|---|---|
| D15S541 | (IR39 R, F) | ab | ab | **b** | Christian *et al.*, 1995 |
| D15S542 | (A124A3 R, F) | ab | ab | **b** | Christian *et al.*, 1995 |
| D15S543 | (ML34 R, F) | ab | b | b | Christian *et al.*, 1995 |
| D15S11 | (43RCA R, F) | a | ab | ab | Mutirangura *et al.*, 1993 |
| D15S210 | (210 R, F) | a | a | a | Malcolm and Donlon, 1994 |
| D15S113 | (LS6-1CA R, F) | ab | cd | **ac** | Mutirangura *et al.*, 1993 |
| GABRB3 | (155-CA R, F) | ab | b | **ab** | Glatt *et al.*, 1994 |
| GABRA5 | (A5-39 R, F) | ab | b | b | Glatt *et al.*, 1994 |
| D15S217 | (217 R, F) | ac | ab | **ac** | GDBID600-687-877 |

*Bold indicates exclusion of paternal UPD as the genotypes of the fetus and the father are different.

the Y heterochromatic region (data not shown) and CBG banding revealed a dicentric chromosome 15 (Fig. 1B). FISH was performed to determine if all the extra material on chromosome 15p was derived from the Y chromosome. DYZ3, a biotin-labelled Y-centromere-specific sequence (Oncor), and a fluorochrome-labelled whole Y-chromosome paint probe (WCP-Y, Vysis) were used. Hybridization and detection conditions of the DNA probes were performed according to the manufacturer's specifications and to those described previously (Knoll and Lichter, 1994). The biotinylated probe was detected with avidin–FITC (fluorescein isothiocynate) and the whole chromosome paint was directly labelled with spectrum orange and did not require detection. The cells were counterstained with DAPI (4′,6-diamidino-2-phenylindole) (0·1 $\mu$g/$\mu$l) and/or propidium iodide (0·2 $\mu$g/$\mu$l) and mounted in antifade (Vectashield, Vector Laboratories). Hybridizations were viewed with an epifluorescence microscope equipped with dual band (FITC/Texas Red; Omega Optical) and triple band pass filter sets (FITC/Texas Red/DAPI; ChromaTech) for the detection of FITC and spectrum orange/DAPI, respectively. A standard single band pass filter (Zeiss) was used to view the DAPI counterstain. Hybridization with DYZ3 revealed the presence of a Y-chromosome centromere on the 15p+ chromosome (Fig. 1C) and hybridization with WCP-Y showed that all extra material on the 15p+ was derived from the Y chromosome (Figs 1D and 1E).

### *Microsatellite analysis*

Genomic DNA was extracted (Puregene, Gentra) from peripheral blood lymphocytes of the parents and from cultured chorionic villus cells of the fetus. The DNAs were amplified by the polymerase chain reaction (PCR) at 15q11q13 loci from within the AS/PWS chromosomal region. The loci with corresponding primer pairs are listed in Table I. PCR cycling parameters are referenced in Table I. Following amplification, electrophoresis of the samples through a 6 per cent polyacrylamide urea gel was performed (Albright and Slatko, 1994). The gel was dried and autoradiographed overnight at room temperature.

## RESULTS

Table I shows the results of microsatellite analysis. Nine loci were examined. Five were informative and showed that the fetus and the father had different genotypes. Locus D15S113 was completely informative (Table I) with the mother heterozygous for alleles *a* and *b* and the father heterozygous for alleles *c* and *d*. The fetus had inherited allele a from the mother and allele *c* from the father. This finding ruled out paternal UPD. At locus D15S217 (Fig. 2A), the mother was heterozygous for alleles *a* and *c*, the father was heterozygous for alleles *a* and *b*, and the fetus had inherited alleles *a* and *c*. This result alone was not informative as to parental origin of allele *a*, but in combination with the cytogenetic result it showed that the fetus had inherited allele *a* from the father and allele *c* from the mother. Allele *a* represents the dic(Y;15) chromosome. At D15S541, both the father and the mother were heterozygous for alleles *a* and *b*, while the fetus was homozygous for allele *b* (Fig. 2B). Again, by combining the cytogenetic data with the molecular data, paternal

 

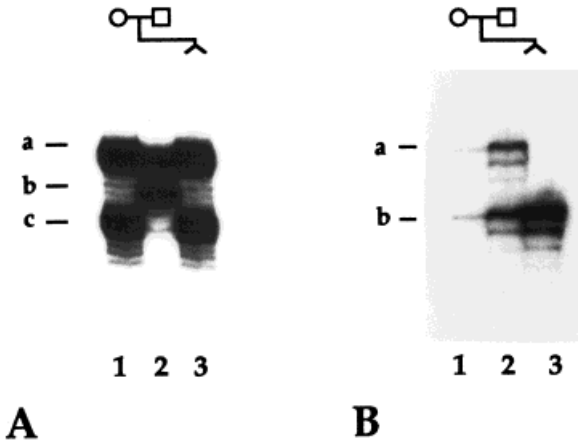


Fig. 2—Microsatellite analysis at (A) D15S217 and (B) D15S541. Maternal DNA is in lane 1 (underloaded in B), paternal DNA is in lane 2, and fetal DNA is in lane 3. Shadow bands are evident at each locus

UPD was excluded. Similar results showing a difference in paternal and fetal genotypes were found for loci D15S542 and GABRB3 (see Table I).

## DISCUSSION

The case presented showed a fetus and a father that shared the same karyotype, 45,X,dic(Y;15) (q11.23;p11.1). A Y;15 translocation resulting in a dicentric chromosome is rare (Gal *et al.*, 1987). This translocation excludes the Yq heterochromatic region, whereas the more common Y;15 translocation is monocentric and contains the Yq-heterochromatic region (Alitalo *et al.*, 1988). In stable dicentric chromosomes, one of the centromeres is thought to become inactivated and thereby prevent chromosomal breakage in subsequent cell divisions (Page *et al.*, 1995; Sullivan and Schwartz, 1995). Depending on which centromere is active in this (Y;15) translocation, different aberrant segregation products are possible. If the Y-chromosome centromere is inactive, abnormal segregation of the sex chromosomes may result, with half of the sperm cells having both sex chromosomes (23,X,dic[Y;15]) and the other half having no sex chromosomes. This aberrant segregation is, in part, due to the pairing and recombination at the distal short arms of the X and Y chromosomes where homology exists. Fertilization of these gametes with a normal female gamete (23,X) would result in 46,XX,dic(Y;15) (Klinefelter syndrome) and 45,X (Turner syndrome) progeny, respectively.

If the chromosome 15 centromere is inactive on the translocated chromosome, then abnormal segregation of the chromosome 15 would result in one gamete with both paternal chromosome 15s and one gamete with no paternal 15 chromosome. Resulting fertilization with a normal female gamete would result in 46,X,dic(Y;15),+15 and 45,XX, − 15, respectively. Neither zygote is viable unless one of the chromosome 15s is lost from the trisomic state or duplicated in the monosomic zygote. Reduction of trisomy to disomy would result in UPD in one-third of cases and correction of monosomy to disomy would result in UPD in all cases (Spence *et al.*, 1988). Paternal UPD results in Angelman syndrome and maternal UPD results in Prader–Willi syndrome, respectively. Biparental inheritance of chromosome 15 (i.e., one chromosome from each parent) is required for normal development.

Centromere activity cannot be determined by standard cytogenetic methods. However, based on the observed sex chromosome aneuploidy in the previous pregnancy and the molecular findings in this pregnancy, it seems likely that the chromosome 15 centromere was active in the translocated chromosome 15. Parental origin of the normal chromosome 15 was not determined in the previous pregnancy but was determined in this pregnancy. DNA polymorphisms at five of the nine 15q11q13 loci revealed different DNA profiles between the father and the fetus. By combining the cytogenetic and molecular data, paternal UPD of 15q11q13 was excluded and biparental inheritance of chromosome 15 was demonstrated. The findings from both pregnancies may reduce the risk for chromosome 15 UPD in subsequent pregnancies but molecular exclusion of UPD remains as a recommendation.

The chromosomal translocation in this family illustrates one example where chromosome 15 UPD testing is relevant and should be performed. Pseudomosaicism or mosaicism of trisomy or monosomy chromosome 15, Robertsonian translocations, reciprocal translocations, and isochromosomes are other examples where prenatal UPD testing of chromosome 15 is indicated. UPD testing is not limited to chromosome 15 abnormalities and should be utilized when other imprinted chromosomes are involved in a structural or numerical abnormality or when there is a

 

defined risk for autosomal recessive disease due to isodisomy. To date, other imprinted chromosomes include chromosomes 7 (maternal), 11 (paternal), and 14 (maternal) (for a review, see Ledbetter and Engel, 1995). UPD testing is most frequently performed by DNA polymorphism analysis but recent findings have shown that interphase FISH cytogenetics is useful in UPD detection of an imprinted region (White *et al.*, 1996). As a result, future UPD testing of an imprinted region will be performed by scoring allele-specific replication patterns on interphase cells from preparations made for routine chromosomal analysis.

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