ELECTRONIC LETTER

Partial hexasomy 15pter→15q13 including *SNRPN* and D15S10: first molecular cytogenetically proven case report

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Isodicentric chromosomes 15 (idic(15) or inv dup(15) chromosomes) are the most common supernumerary marker chromosome (SMC) in humans.[1] Buckton *et al*[2] found that 0.24% of newborns had a SMC, of which 50% could be described as idic(15). The presence of such chromosomes is usually associated with mental retardation, developmental delay, epilepsy, and behavioural problems like autism.[3] It is possible that the clinical severity depends on the size of the SMC and its euchromatic material, for example, almost no clinical signs were detectable in a case with idic(15)(pter→q12),[4] while a severe phenotype including all typical clinical signs was present in a patient with the karyotype 47,+idic(15)(pter→q13).[3 5 6] However, there are also reports which show an inconsistent relationship between marker size, gene dosage, and severity of the phenotype.[7 8] In the study of Mignon *et al*,[8] in all 16 cases studied the derivative chromosomes 15 were of maternal origin with an identical methylation profile, and neither imprinting nor methylation could explain the phenotypic variability. Partial trisomy of 15q11-q14 resulting from intrachromosomal duplication of chromosome 15q11-q14 has also been reported.[9 10]

Here we describe the first molecular cytogenetically proven case of a girl with the karyotype 48,XX,+2 idic(15)(pter→15q13).ish 15q13(SNRPN×6, D15S10×6). The clinical details and the results of the molecular and molecular cytogenetic studies are presented and discussed.

Key points

- We report on a case of a partial hexasomy 15pter→15q13 including the loci *SNRPN* and D15S10 as shown by different fluorescence in situ hybridisation (FISH) methods.
- The newborn girl presented with postaxial polydactyly of both hands and one foot, muscular hypotonia, and a heart defect. She developed seizures and at the age of 3 years she had severe developmental delay, microcephaly, and pachygyria.
- The karyotype was described as 48,XX,+2mar after GTG banding. The origin of the two additional identical supernumerary marker chromosomes (SMC) was characterised using a centromere specific multicolour FISH (cenM FISH) approach. Multicolour banding (MCB) and two probes specific for the Prader-Willi-syndrome chromosome region in 15q13 defined the derivative chromosomes as idic(15)(pter→q13).
- PCR based methylation analysis at the *SNRP* locus suggested that the isodicentric chromosomes 15 were of maternal origin.

MATERIALS AND METHODS

Clinical report

The girl was born after 41 weeks of an uneventful pregnancy to a non-consanguineous German couple. At birth, the mother was 27 years and the father 31 years old. Anthropometric measurements at birth were within the normal range (length 50 cm, weight 3520 g, head circumference 34 cm). The girl had postaxial polydactyly of both hands (fig 1A(a)) and the right foot (fig 1A(b)), a haemangioma on the back (5 × 5 cm), and muscular hypotonia. The heart showed patent ductus arteriosus and an open foramen ovale. From the age of 3 days the girl developed seizures that are still difficult to prevent. At the age of 3¼ years she presented with severe developmental delay and microcephaly (44 cm, –4 SD) (fig 1A(c)). Height and weight were within the normal range for her age. In addition, she has strabismus, hyperopia, and coxa vara on both sides. MRI scan of the skull showed pachygyria.

Cytogenetics and molecular cytogenetics

Cytogenetic and molecular cytogenetic studies were performed on chromosomes derived from peripheral blood. Chromosome preparations and GTG banding were performed according to standard techniques[11] (fig 1B). Fluorescence in situ hybridisation (FISH) including RNase and pepsin pretreatment, denaturation of the slides, and addition of the probe to the sample were performed according to standard protocols.[12] CenM FISH was performed as described previously[13] with the modification that as 24th probe a probe specific for the short arms of all acrocentric chromosomes was added to the cenM FISH probe mix (fig 1C(a)); the probe called midi54 is described in Mrasek *et al*.[14] The cenM54 FISH results (fig 1C(b)) have been verified using single colour FISH with two commercially available centromeric probes for chromosome 15 (alpha satellite probe and satellite III DNA probe, Vysis (fig 1D). Additionally, the *SNRPN* and the D15S10 specific probes in 15q13 (Vysis, fig 1D) and the multicolour banding (MCB) probe set for chromosome 15[15 16] were applied (fig 1E). The results were evaluated on a fluorescence microscope equipped with a CCD camera and an image analysis system (MetaSystems, Altlussheim, Germany).

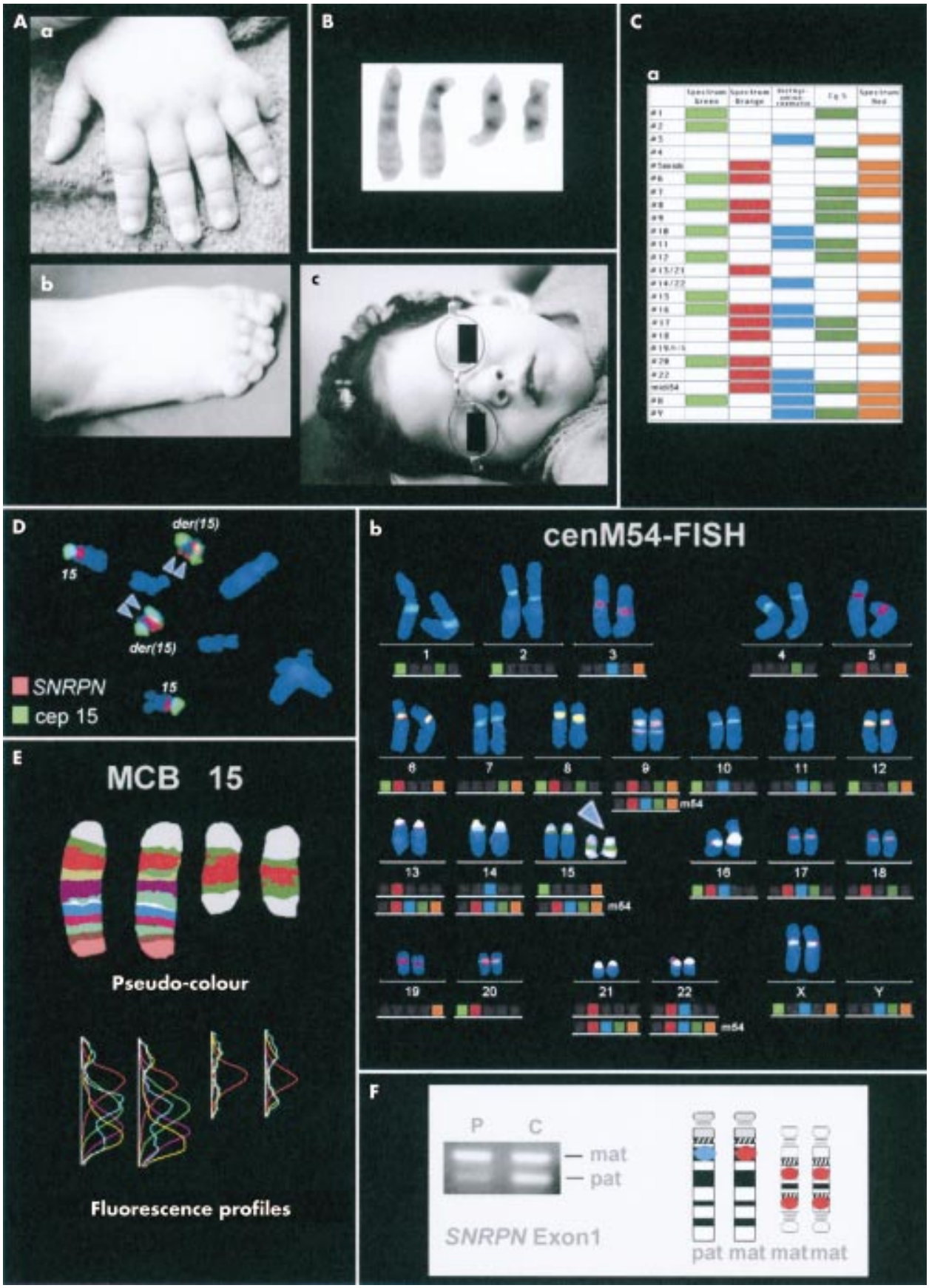
Methylation analysis

Methylation analysis for the *SNRP* exon 1 region was performed using methylation specific PCR.[17] The PCR products were analysed on a 2% agarose gel (fig 1F).

RESULTS

Cytogenetic analysis in the severely retarded girl (fig 1A(c)) showed a karyotype 48,XY,+2mar (fig 1B). The small markers were present in all lymphocyte metaphase spreads analysed. The markers could not be detected in either parent.

A new variant of the centromere specific multicolour FISH (cenM FISH), cenM54 FISH, applied to metaphases of the



child showed in one single hybridisation that a partial hexasomy of 15pter→15q12-14 was present, as the SMC were characterised as dicentric derivatives of chromosome 15 including two p arms (fig 1C). FISH with a commercially available alphoid probe and satellite III probe for chromosome 15 (Vysis) confirmed the result (fig 1(D)). Additionally, the *SNRPN* specific probe in 15q13 (Vysis[4]), the probe D15S10 (data not shown), and the multicolour banding (MCB) probe set for chromosome 15[15 16] (fig 1E) showed that the SMC could be described as idic(15)(pter→15q13).ish 15q13 (SNRPN×6,D15S10×6).

The methylation analysis at the *SNRP* locus allows determination of the ratio of the PCR product of the methylated maternal allele compared to the PCR product of the unmethylated paternal allele. The overexpression of the methylated maternal PCR product suggests that the derivative chromosomes 15 are of maternal origin (fig 1(F)).

DISCUSSION

To the best of our knowledge this is the first report of a case with a karyotype 48,XX,+2 idic(15)(pter→15q13).ish 15q13 (SNRPN×6,D15S10×6). The two isodicentric chromosomes 15 were characterised by GTG banding (fig 1B), by cenM54 FISH (fig 1C(b)), by multicolour banding (MCB),[15 16] (fig 1E) and by locus specific probes for 15q13 (fig 1D). Apart from the common cases with one idic(15),[1 3 5 6] corresponding to a partial trisomy or tetrasomy of the Prader-Willi and Angelman syndrome critical region (PWACR), one mosaic case with two idic(15) with one copy of *SNRPN* and *GABRB3* each, leading to a partial tetrasomy of the PWACR,[18] and two non-mosaic cases have been described so far with partial pentasomy of 15q11-q13 (karyotype: 47,XX,dup(15) (q11q13),+idic(15) (pter→q13)).[19 20] Robinson *et al*[21] described a case with 48,XX,+2,inv dup(15)(q13). However, they could only show the presence of four copies of the PWACR. Cases described in the time before molecular cytogenetic characterisation was possible[1] might have had six copies of the PWACR as well. However, to prove this such cases would have to be re-examined.

A maternal origin of the two derivative chromosomes 15 of the present case is most likely, as described for all similar clinical cases with tetrasomy 15q13.[8 18 19 21–23] Mechanisms for idic(15) formation have been proposed previously.[21 24] For the establishment of a dicentric chromosome, a crossing over event in meiosis I followed by a non-disjunction in meiosis II has been suggested by Martinsson *et al*.[24] The presence of two identical isodicentric chromosomes can be explained by a non-disjunction event in early embryogenesis.

In 1993, Robinson *et al*[21] postulated that the clinical severity of cases with idic(15) is associated with the dosage of the PWACR rather than with differences in the extent of the duplicated segment. This suggestion is confirmed by the clinical signs of the present case, which are in accordance with a very severe type of the "inv dup(15) syndrome".[25] However, the polydactyly is unique in our patient and has not been described in patients with SMC(15) so far.

Figure 1 (A) The left hand (a) and the right foot (b) of the female patient showing postaxial hexadactyly and (c) the patient's head showing microcephaly. (B) GTG banding results of the two normal and the two derivative chromosomes 15, karyotype: 48,XX,+2mar. (C) The identical two supernumerary marker chromosomes (SMC) could be identified as derived from chromosome 15 cenM54 FISH (b). As in the five colour overlay the resulting colours in some cases look very similar, below each chromosome pair the corresponding colour combinations are given. They were analysed in the different colour channels using MetaSystems software. The labelling scheme for cenM FISH is depicted in (a). The probe midi54 (m54) is specific for the short arms of all human acrocentric chromosomes; as outlined in Mrasek *et al*[14] the chromosomal bands 9p12 and 9q13-21.1 are stained by midi54 too. The SMC are identified as dicentric der(15) including two short arms of the acrocentric chromosomes (arrowhead) applying the cenM54 probe set. (D) Results of a two colour FISH experiment hybridising simultaneously commercially available centromeric probe for chromosome 15 (Vysis, SpectrumGreen) and the *SNRPN* probe specific for the Prader-Willi and Angelman syndrome region (Vysis). The result of cenM FISH was verified by the centromeric probe and the PWS specific probes showed that a hexasomy of this region was present. (E) Multicolour banding (MCB) results of the two chromosomes 15 and the two SMC. Pseudo-colour and fluorescence profiles clearly indicate the origin of the chromosomal material on the idic(15) chromosomes. The short arms are pseudo-coloured in grey. (F) Results of the analysis of allelic methylation differences in 15q11-q13. The maternal (mat) and paternal (pat) allele are present in an internal control (C) in equal proportions, while in the patient (P) the maternal allele is clearly over-represented. Thus, maternal origin of one chromosome 15 and the two idic(15) is suggested.

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