Intrachromosomal triplication of 15q11–q13

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Abstract

A 7 year old girl with intrachromosomal triplication 46,XX,−15, + der(15)(pter→q13::q13→q11::q11→qter) resulting in tetrasomy of 15q11–q13 is reported. Fluorescence in situ hybridisation confirmed that the tetrasomic region included the entire segment normally deleted in Prader-Willi and Angelman syndrome patients, and breakpoints were similar to those reported in two tandem duplications of 15q11–q13. The middle repeat was inverted, suggesting a possible origin through an inverted duplication intermediate. Microsatellite analysis showed that the rearrangement was of maternal origin and involved both maternal homologues. Clinical findings included multiple minor anomalies (a fistula over the glabella, epicanthic folds, downward slanting palpebral fissures, ptosis of the upper lids, strabismus, a broad and bulbous tip of the nose, and small hands and feet), motor and mental retardation, a seizure disorder, and limited verbal abilities. In addition, immunological examination disclosed a selective immunodeficiency. The overall phenotype did not clearly resemble that of cases with tetrasomy 15pter→q13 associated with an extra inv dup(15)(pter→q13;q13→qter) chromosome. The latter aberration causes more severe mental deficit and intractable seizures, but less marked phenotypic alterations, although some overlap in mild facial dysmorphic features is present. A number of features common to Angelman syndrome were also observed in the patient.

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A deletion of chromosome 15q11–q13 is a relatively frequently occurring chromosome rearrangement and is observed in the majority of Prader-Willi syndrome (PWS) and Angelman syndrome (AS) patients. Apparent duplications of this region detected by cytogenetic analysis have also been observed and were associated with a variety of phenotypes including apparently normal, AS, PWS, and an abnormal phenotype not resembling either AS or PWS. Molecular analysis confirmed a maternally derived tandem duplication of this region in a patient with features similar to AS and a tandem duplication of paternal origin in a patient with atypical PWS. These patients, however, had a milder phenotype than is typical of PWS or AS.

Tetrasomy of the PWS/AS deletion region is commonly observed in the form of inv dup(15) chromosomes. The phenotype of such patients is also variable, but typically includes normal growth, severe to profound mental retardation, abnormal EEG, epilepsy refractory to therapy, hypotonia, poor motor coordination, and no or only mild dysmorphic features.

Only two cases of an intrachromosomal triplication of 15q11–q13 have been reported previously, although a triplication would be difficult to distinguish from a duplication without fluorescence in situ hybridisation (FISH). The clinical features of a patient presented here with an intrachromosomal triplication of 15q11–q13 should assist in the identification of similar patients. Molecular analysis of the tetrasomic region adds information important to our understanding of the mechanism of duplication, deletion, and inv dup(15) formation in this frequently rearranged chromosomal region.

Case report (table 1)

This 7 year old girl is the third of four children born to healthy, unrelated parents; the father was 34 and the mother 33 years old at her birth. The pedigrees are unremarkable. Pregnancy was uneventful, as was delivery at 40 weeks. Birth weight was 3500 g and length 50.5 cm. The early postnatal course was normal, and she was discharged from hospital with her mother after one week. However, at a paediatric check up at 3 months of age, hypertonia, spasticity, and poor head control were noticed. Developmental delay was confirmed at another examination at 8 months. The first epileptic fit occurred at the age of 5 years 10 months, when she was found unconscious in her bed one morning. She had a second attack several months later, again in the morning, after the evening antiepileptic medication had been forgotten. She began walking at 30 months and at 7 years is still incontinent of urine day and night. Her vocabulary is restricted to about 100 words. Recurrent upper and lower respiratory tract infections have occurred from infancy onwards and prompted immunological investigations.
Clinical examination at the age of 7 years (fig 1) showed the following: height 1·14 m (3rd to 10th centile), weight 21 kg (25th centile), OFC 50·5 cm (10th to 25th centile), hand length 12·0 cm (3rd centile), foot length 15 cm (<3rd centile), and inner canthal distance 3·2 cm (about 75th centile). She displayed the following minor anomalies: low frontal hair line, a fistula over the glabella cranial to the inner canthal line from which occasionally a white fluid was secreted, a prominent nasal bridge, bilateral inner epicanthic folds, downward slanting palpebral fissures, ptosis of the upper lids (more marked on the right side), exotropia, a broad and bulbous tip of the nose, small irregularly positioned teeth, the upper incisors being lost accidentally, prominent lateral palatine ridges, and mild pectus excavatum. The mouth was not large, the mandible was of normal size, and extremities were normal including nails and palmar creases.

Her gait was unsteady, but on neurological examination there was neither true ataxia nor clonus, and reflexes were normal. When asked, the mother confirmed jerky movements and inappropriate laughter, but this was not seen at examination. She is aggressive against herself and others and hyperactive. Psychiatric examination disclosed autistic features in addition to the above mentioned characteristics.

CT scan showed normal brain structure. An EEG showed a slowing of background activity (polymorphic delta waves) and a loss of the drug induced beta activity over the right hemisphere.

Immunological examinations were performed because of frequent respiratory infections which occurred from infancy onwards. Serum levels of IgG, IgM, and IgA were determined by nephelometry and showed IgG levels of 4·85 and 6·95 g/l, IgA levels of <0·08 and 0·13 g/l, and IgM levels of 0·27 and 0·44 g/l at 5 1/2 and 11·5 years of age, respectively. The IgG subclass levels were determined by radial immunodiffusion with use of subclass specific polyclonal antibodies (Central Laboratory of the Netherlands’ Red Cross) and showed normal values for age for IgG1, IgG2, and IgG4, but undetectable IgG3 levels. IgE was present at a normal level for age (16 kU/L). Before and after Haemophilus influenza type B (PRP-T conjugate vaccine) and pneumococcal (Pneumovax®) vaccinations, no specific antibody production could be detected as measured by line immunobinding assay.16 Cellular immunity was assayed in vitro and showed no abnormality. T and B cell phenotypes were normal, as were T cell proliferation tests after mitogenic (concanaalin A, phytohaemagglutinin, pokeweek mitogen) and antigenic stimulation with antigens to which the patient had been exposed (purified protein derivative, tetanus toxoid, candidin). A defect in the antibody production against polysaccharide antigens was concluded from the immunological studies in this patient.

Methods

CYTOGENETICS AND FISH

Cytogenetic examinations were performed on blood lymphocyte cultures of the patient and her parents using standard techniques. Preparations were GTG and CBG stained. FISH was performed on metaphase chromosome preparations following the protocol from Driesen et al11 (for single colour) or Arnoldus et al10 (for bicolour).

Single colour FISH in the patient was performed using two cosmid probes for IR4-3R (D15S11) and GABRB3 available from Oncor Inc (following the Oncor protocol). Yeast artificial chromosome (YAC) probes in and flanking the PWS/AS common deletion interval were also used: A124A3 (IR39;D15S18), 149A9 (IR10-1;D15S12), and B94H7 (CMW-1;D15S24).13 Biotinylated YAC DNA (125 ng) was used in 11 µl hybridisation solution. In
addition, a chromosome 15 library (pBS-15) obtained from Lawrence Livermore Laboratories was hybridised. For bicolour FISH, 500 ng of A124A3 or 149A9 DNA was labelled with digoxigenin using a nick translation kit from Boehringer Mannheim. Hybridisation was performed for four days using a mix of (1) 8 µl of Oncor 'PWS-region A' (D15S11) biotinylated probe plus 125 ng of digoxigen labelled 149A9 DNA in a total volume of 11 µl, or (2) 8 µl of Oncor 'PWS-region B' (D15S11) biotinylated probe plus 125 ng of digoxigen labelled A124A3 DNA in a total volume of 11 µl.

A bicolour image was obtained with a Leitz Diaplan fluorescence microscope coupled to a cooled CCD camera (Photometrics, Tucson, AZ). A Macintosh Quadra 800 computer was used for camera control. Image acquisition was performed using the software package IPLab Spectrum (Signal Analytics).14

A lymphoblastoid cell line is not yet available from the patient.

MOLECULAR POLYMORPHISM ANALYSIS
Six dinucleotide repeat polymorphisms were used, including D15S11, D15S113, GABRB3, D15S13,15 and D15S97 (Genome data base). PCR was performed on a Perkin-Elmer-Cetus thermocycler using 57°C annealing temperature and 30 cycles. In addition, the probes ML34 (D15S9), IR10-1 (D15S12), and CMW1 (D15S24) (Genome data base) were hybridised by Southern blot analysis.

IMMUNOLOGICAL INVESTIGATIONS
Serum levels of IgG, IgA, and IgM were determined by nephelometry, and the IgG subclass levels were determined by radial immunodiffusion with the use of subclass specific polyclonal antibodies (Central Laboratory of the Netherlands' Red Cross). Anti-tetanus and anti-diphtheria IgG were measured by Elisa, and anti-polysinosyl-ribitol-phosphate (Haemophilus influenzae type B antigen) and

Figure 2 (A–D) FISH results in metaphases of the patient. (A–C) Biotin labelled probe detected via FITC and counterstained with PI. (A) ONCOR PWS region B (GABRB3) probe showing three distinct signals on one chromosome. (B) YAC probe A149A9 (D15S12): the middle signal on the triplication chromosome appears to be closer to the proximal than to the distal signal. (C) YAC probe A124A3: the middle segment on the triplication chromosome is closer to the distal than to the proximal signal. Metaphases A and C were photographed with a Chroma triple band pass filter. (D) CCD image of a bicolour FISH using biotin labelled ONCOR PWS region A (green-yellow) and digoxigen labelled YAC A149A9 (red). The abnormal 15 shows from centromere to telomere a single yellow-double red-double yellow-single red signal indicating an inverted orientation of the middle repeat (as shown in diagram form in fig 4).
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Table 2: Molecular results of the patient and parents.

<table>
<thead>
<tr>
<th>Locus (probe/enzyme)</th>
<th>Father</th>
<th>Patient</th>
<th>Mother</th>
</tr>
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<tr>
<td>D15S9 (ML34/ScaI)</td>
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<td>1222</td>
<td>12</td>
</tr>
<tr>
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<tr>
<td>D15S24 (CMW-1/ScaI)</td>
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<td>ab</td>
</tr>
<tr>
<td>D15S86 (ms620/TaqI)</td>
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Results

Cytohensetics and FISH

GTG banding analysis showed a 46,XX,15q+ karyotype which was interpreted to be a duplication of the 15q11-q13 region. On CBG banding, no second centromere could be shown. FISH of a chromosome 15 library confirmed that the 15q+ chromosome was completely derived from chromosome 15. FISH of the cosmids for D15S11 and GABRB3, and YACs A124A3 and A149A9 each showed three distinct signals on the 15q+ chromosome and one signal on the normal chromosome 15 (fig 2A). B4H7 clearly showed two signals on the rearranged chromosome but it could not be determined if three signals were present or not. The distances between the signals did not appear to be equal, as would be expected if the duplications had occurred in direct orientation: for 149A9, the middle signal was closer to the proximal signal from the centromere than to the distal signal (fig 2B); for A124A3, the middle signal was closer to the more distal signal (fig 2C). We infer this to mean that the middle repeat was inverted in orientation. Bicolour FISH confirmed three copies of all probes hybridised within the PWS deletion region, and also confirmed that the middle segment is inverted (fig 2D).

Molecular polymorphism analysis

Molecular analyses of microsatellite polymorphisms within 15q11-q13 showed inheritance of one paternal plus two different maternal alleles at multiple loci in the patient (table 2) (fig 3). She showed three distinct alleles for the D15S9, D15S11, GABRB3, and D15S97 polymorphisms with results indicating that one maternal allele was present in two copies and a second in one copy. Normal biparental inheritance of a distal chromosome 15q marker (D15S86) was seen. These results indicate that the triplication was maternal in origin and involved the two different maternal chromosomes 15. An idiogram of the normal and abnormal chromosomes with indication of the orientation of the three PWS/AS regions in the latter is given in fig 4.

Figure 3: Microsatellite results for D15S11 and GABRB3 show inheritance of two different maternal alleles and one paternal allele.

Figure 4: Idiogram of the normal and triplicated chromosome 15 in the patient indicating the orientation of probes detecting two segments within the PWS/AS critical region (more proximal: PWS-A; more distal: A149A9): "normal" in the normal left homologue and in the proximal and distal region of the triplication right homologue, inverted in the middle region of the triplication homologue.
IMMUNOLOGICAL INVESTIGATIONS

IgG levels were 4.85 and 6.95 g/l, IgA levels <0.08 and 0.13 g/l, and IgM levels 0.27 and 0.44 g/l at 5 years 1 month and 5 years 9 months of age, respectively. IgG1, IgG2, and IgG4 values were in the normal range for age (16 kU/l). The antibody production after diphtheria and tetanus toxoid booster showed normal levels of specific IgG. Before and after Haemophilus influenzae type B (PRP-T conjugate vaccine) and pneumococcal (Pneumovax®) vaccinations, no specific antibody production could be detected in the IgG1 and IgG2 subclasses. Cellular immunity did not show any abnormality: T and B cell phenotypes were normal, as were T cell proliferation tests after mitogenic and antigenic stimulation with antigens to which the patient had been exposed. These results indicate a selective defect in the antibody response to polysaccharide antigens without associated T cell immunodeficiency.

Discussion

CLINICAL FEATURES

It is interesting that the patient showed many features similar to Angelman syndrome (AS), as has also been reported for a patient with a maternal duplication of 15q11-q13.5 Compared to characteristic AS patients, however, her mental and motor delay is milder, she lacks obvious ataxia, and has an immunodeficiency which has not been described in AS patients. In addition, her minor dysmorphic features differed from those commonly seen in AS; in particular, she showed a glabellar fistula, but she did not show the wide mouth and prominent mandible characteristic of AS patients of her age. Furthermore, although she has had two epileptic fits, neither the severity of clinical expression of her seizure disorder nor the EEG fit that commonly seen in AS. The phenotype of AS is, however, variable and some features, such as ataxia and epilepsy, seem to be milder in AS patients with 2 copies of 15q11-q13, by paternal UPD,13 than in deletion patients, with just one copy of this region.17

Some similarities also exist between the present patient and inv dup(15) patients, who also have tetrasomy of the 15q11-q13 region. Our own investigations of 10 patients with 47, + inv dup(15)(pter→q13:q13→pter) by FISH and microsatellite analysis confirm that the tetrasomy region is similar to that in the present patient, and is always maternal in origin with the involvement of both different maternal chromosomes 15q11-13 haplotypes7 (unpublished results). Although tetrasomy in the inv dup(15) patients additionally includes the region between the centromere and 15q11, this region is thought to contain only heterochromatin, and patients with 47, + inv dup(15)(pter→q11:q11→pter) usually have a normal phenotype. In addition, PWS patients with unbalanced translocations resulting in loss of 15pter-q13 do not clinically differ from those with the "common" 15q11-13 deletion. The distal boundary of the duplicated segments in the inv dup(15) patients is similar to, and, in some cases, more proximal to that in the case described here and hence cannot account for the phenotypic differences. It is probable, therefore, that the position and orientation of the duplicated 15q11-q13 region has an important influence on the phenotype.

Recurrent infections and immunodeficiency have not so far been reported in AS nor in PWS patients. Also, no humoral selective immunodeficiency has been described as involving this region of chromosome 15. Information about chromosomal aberrations in patients with impaired antibody responses to polysaccharides is lacking,18 and they would be highly unlikely to occur as these patients tend to have a normal phenotype. The mechanism of the deficiency is unknown: however, if the position and the orientation of the triplicated segment 15q11-q13 does indeed influence the phenotype, one could also hypothesise that the immunodeficiency is the result of such a position effect.

MECHANISM OF ORIGIN

The triplicated (tetrasomic) region and parental origin were similar in our case and previously reported inv dup(15)(q13) cases. As, in the present patient, the middle repeat was inverted in orientation, origin through a double unequal crossover is unlikely. We suggest as just one possibility that the triplication was initiated via an unstable, dicentric inv dup(15)(pter→q13;q13→pter) chromosome. Such chromosomes occur relatively frequently and are normally maternal in origin. If the second centromere was not inactivated immediately after formation, a broken chromosome would result at the subsequent cell division. Such a broken chromosome would contain a direct and inverted repeat and would tend to recombine with the remaining normal chromosome 15. Such a mechanism has been proposed to explain amplification of the dihydrofolate reductase (DHFR) gene in Chinese hamsters cells.19 In this latter case, a high frequency of dicentric chromosomes and chromosomes with sister chromatids fused at their termini was found in association with megabase long amplifications (from two to multiple copies) of DNA containing the DHFR locus. As no mosaicism was found in the present case, which could elucidate better the mechanism involved, it is impossible to prove the origin.

The identification of this triplication case and two other recently reported cases7 indicates that amplification of this region may not be rare and highlights the instability of this proximal 15q segment. The lack of similar published reports of triplication of 15q11-q13 is presumably because of the inability to demonstrate the aberration before FISH examination. It is therefore possible, if not probable, that some of the cases previously reported to have duplication of 15q11-q13 or a similar segment instead have a triplication of this region.4

In summary, we report a case of intrachromosomal triplication of proximal chromosome 15q which results in tetrasomy for 15q11-13. FISH examination indicated that one chromosome 15 contained three se-
Intrachromosomal triplication of 15q11–q13 of which the first and third repeat were most likely in direct orientation while the middle one was inverted. The phenotype was milder than that of cases of tetrasomy of a similar segment of 15 owing to formation of an additional inv dup(15) chromosome with respect to mental retardation and seizures, while minor dysmorphic traits were more distinct and immunological dysfunction was observed in our patient. Although several theoretical explanations can be provided, there is no conclusive explanation for the origin of these cases.

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5 Bundey S, Hardy C, Vickers S, Kilpatrick MW, Corbett JA. Duplication of the 15q11–13 region, including the genes for two gamma aminobutyric acid receptors, in a patient with the syndrome of autism, epilepsy and ataxia. Dev Med Child Neurol (in press).


