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Der(22)t(11;22) resulting from a paternal de novo translocation, adjacent 1 segregation, and maternal heterodisomy of chromosome 22

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Abstract

The t(11;22)(q23;q11) translocation is the most frequently identified familial reciprocal translocation in humans. In translocation carriers, 3:1 meiotic segregation with tertiary trisomy can occur resulting in abnormal progeny with the der(22) as the supernumary chromosome. Affected children have a distinct phenotype with multiple anomalies and severe mental retardation. We have identified a child with developmental delay and multiple anomalies consistent with the der(22) phenotype. Cytogenetic analysis showed an abnormal chromosome complement of 47,XX,+der(22)t(11;22)(q23;q11) in all 50 cells analysed. FISH analysis using chromosome 11 and 22 painting probes showed a pattern consistent with a reciprocal translocation of the distal bands 11q23 and 22q11 respectively. Parental karyotypes were normal. RFLP analysis of locus D22S43, which maps above the t(11;22) breakpoint, showed that the der(22) was paternal in origin and indicated that the normal chromosomes 22 were the probable result of maternal heterodisomy. RFLP analysis of locus D22S94, which maps below the t(11;22) breakpoint, also suggested that both normal chromosomes 22 of the child represented the two maternal homologues. Non-paternity was excluded through the analysis of 10 microsatellite markers distributed on 10 different chromosomes and three VNTRs on three different chromosomes. To the best of our knowledge, this is the first reported case of a patient with an abnormal karyotype resulting from a de novo translocation in the paternal germline with probable unbalanced adjacent 1 segregation and maternal non-disjunction of chromosome 22 in meiosis I.

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Key words: der(22)t(11;22); adjacent 1 segregation; maternal heterodisomy.

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The t(11;22)(q23.3;11.2) is the most frequently identified human reciprocal translocation.[1–3] Abnormal offspring of balanced carriers invariably have a tertiary trisomy resulting from 3:1 segregation of the der(22) chromosome. This results in a 47,XX/XY, +der(22)t(11;22)(q23.3;q11.2) karyotype with trisomy for distal 11q and pericentromeric 22. Affected children have a distinct phenotype with multiple anomalies and severe mental retardation.

A balanced carrier for this translocation does produce other types of unbalanced gametes.[4 5] Analysis of the sperm chromosome complements of balanced carriers showed that all forms of 2:2 segregants, including alternate and adjacent 1 and 2, as well as 3:1 segregants, are produced. This indicates that adjacent 1 and 2 segregants are formed but lead to non-viable conceptuses. There are no reports of viable offspring resulting from adjacent 1 and 2 meiotic segregation in a heterozygote for this translocation.

There have been several reported cases of abnormal offspring having inherited both the parental balanced translocation as well as the der(22) as a supernumary chromosome.[6–9] These cases, it has been suggested, are the result of alternate segregation at meiosis I with either meiotic II or postzygotic non-disjunction of the der(22) which, in addition to the presence of the two translocated chromosomes, has resulted in a 47,XX/XY,t(11;22)(q23.3;q11.2), +der(22)t(11;22)(q23.3;q11.2) karyotype. This karyotype has also been postulated to result from a crossover involving the normal chromosome 22 and the der(22) between the centromere and translocation breakpoint, followed by 3:1 segregation of the der(11), der(22), and normal chromosome 22.[10] The clinical phenotype, as expected, is similar to that of the usual unbalanced karyotype resulting in tertiary trisomy of der(22).

We now report a viable case of 47,XX, +der(22)t(11;22)(q23.3;11.2)pat resulting from adjacent 1 segregation in a de novo paternal translocation which has been rescued by complementation with a maternal gamete which underwent meiosis I non-disjunction of the chromosomes 22.

Materials and methods

CYTOGENETIC ANALYSIS AND FLUORESCENT IN SITU HYBRIDISATION

Chromosomes studies were performed on lymphocytes using standard methods.[11] Fluorescent in situ hybridisation (FISH) was performed on metaphase spreads probed with both chromosome 11 and chromosome 22 painting "COATASOME" probes (ONCOR) and the DiGeorge chromosome region/chromosome 22 marker probes (ONCOR).

Table 1 Chromosome location of microsatellite loci analysed

Locus	Chromosome
*D1S158	1
*hTPO	2
*D3S1359	3
MYC	8
*D11S569	11
*vWF	12
D14S245	14
PACAP	18
D21S11	21
*DXS101	X

* Markers used in the calculation for probability of paternity.

DNA AND PROBES

Genomic DNA from the proband and her parents was isolated according to standard methods.[12] Probes used to identify the parent of origin of the normal chromosome 22 and der(22) of the proband have been mapped previously to the long arm of chromosome 22 or 22q (see below). RFLP analysis[12] was used to identify the parent of origin of the normal and der(22) chromosomes of the proband. D22S43, which maps to the cat eye syndrome region at 22q11.2[13] (above the t(11;22) breakpoint), shows a three allele polymorphism (2.9, 3.8, and 4.8 kb) with *Taq*I.[14] D22S94 (Kl-1105) and D22S45 (pH41a), which both map to 22q13.3[15] (well below the t(11;22) breakpoint), show a two allele (6.6 and 5.5 kb)[15] and three allele (2.1, 2.6, and 2.7 kb)[14] polymorphism, respectively, with *Taq*I. A rare variant of 5.4 kb at the D22S94 locus was found in this family.

To confirm paternity of the proband, two sets of probes were used. Three VNTR probes were obtained from the American Type Culture Collection: D1S57 with over eight alleles with *Msp*I[16]; D2S44 with more than 30 alleles with *Msp*I[17]; and D14S13 with over 10 alleles with *Msp*I.[17] Ten polymorphic microsatellite markers, which included a variety of dinucleotide and tetranucleotide repeats distributed on 10 different chromosomes, were also examined.[18] These markers and their chromosomal location are summarised in table 1.

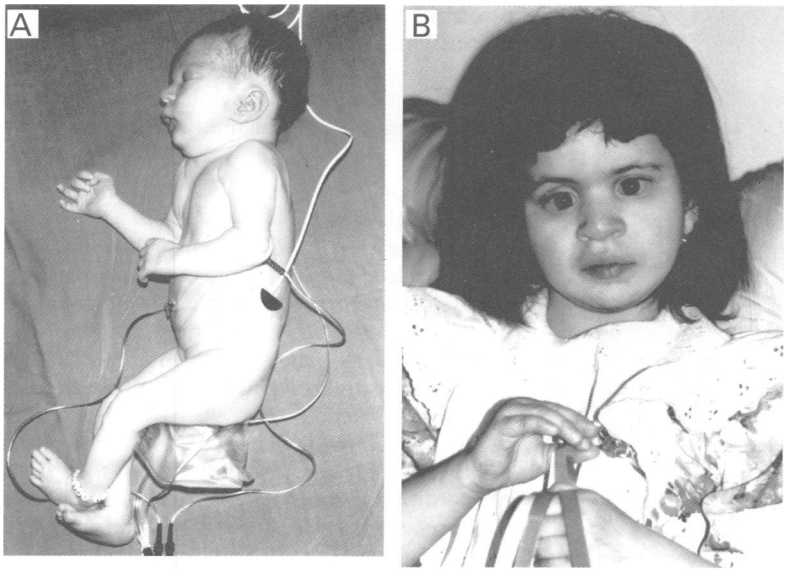

Figure 1 The proband (A) as a newborn (note unusual hands and rocker bottom feet) and (B) aged 8 years.

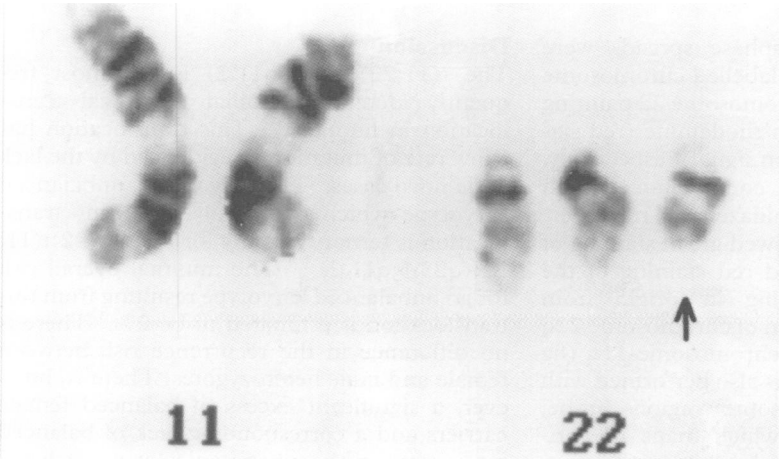

Figure 2 Partial G banded karyotype of the proband. The arrow denotes the der(22) chromosome.

Results

SUBJECT

An 8 year old year female originally presented to the Genetics Clinic as a newborn with multiple anomalies consistent with the der(22)t(11;22)(q23.3;11.2) syndrome (fig 1). She was the 37 week product of a normal pregnancy, labour, and delivery. Birth weight was 2480 g. She was the first child born to a mother who was 24 years of age and a father who was 27 years of age at the time of her birth. She has two healthy sibs. The family history was unremarkable.

Anomalies consisted of mild intrauterine growth retardation or IUGR, a prominent occiput, narrow palpebral fissures, depressed nasal tip, low set ears, preauricular pits and ear tags, high arched palate, micrognathia, flexor contractures of the elbows and knees, dislocated right hip, transitional palmar creases, overlapping toes, and rocker bottom feet. The child had complex cardiac anomalies, confirmed by cardiac catheterisation in the newborn period, which included tetralogy of Fallot, AV canal, and a large PDA. She underwent a Blalock-Taussig shunt at 18 months of age. She walked at 7 years. At $7\frac{1}{2}$ years she was moderately retarded in cognitive development with limited speech, a wide based, spastic gait, and deep cyanosis. She is being considered for further palliative cardiac surgery. All growth parameters were below the 5th centile.

CYTOGENETICS AND FISH

The proband had a female karyotype with 47 chromosomes and a supernumerary marker chromosome. This chromosome appeared to be a der(22)t(11;22)(q23.3;q11.2) (fig 2). All 10 cells examined from the proband had this karyotype. The chromosomes of both parents were normal in all 200 cells examined (100 cells/parent). This excludes a 5% level of mosaicism for the t(11;22) at a confidence limit of 99%. The identification of the supernumerary chromosome was confirmed using FISH chro-

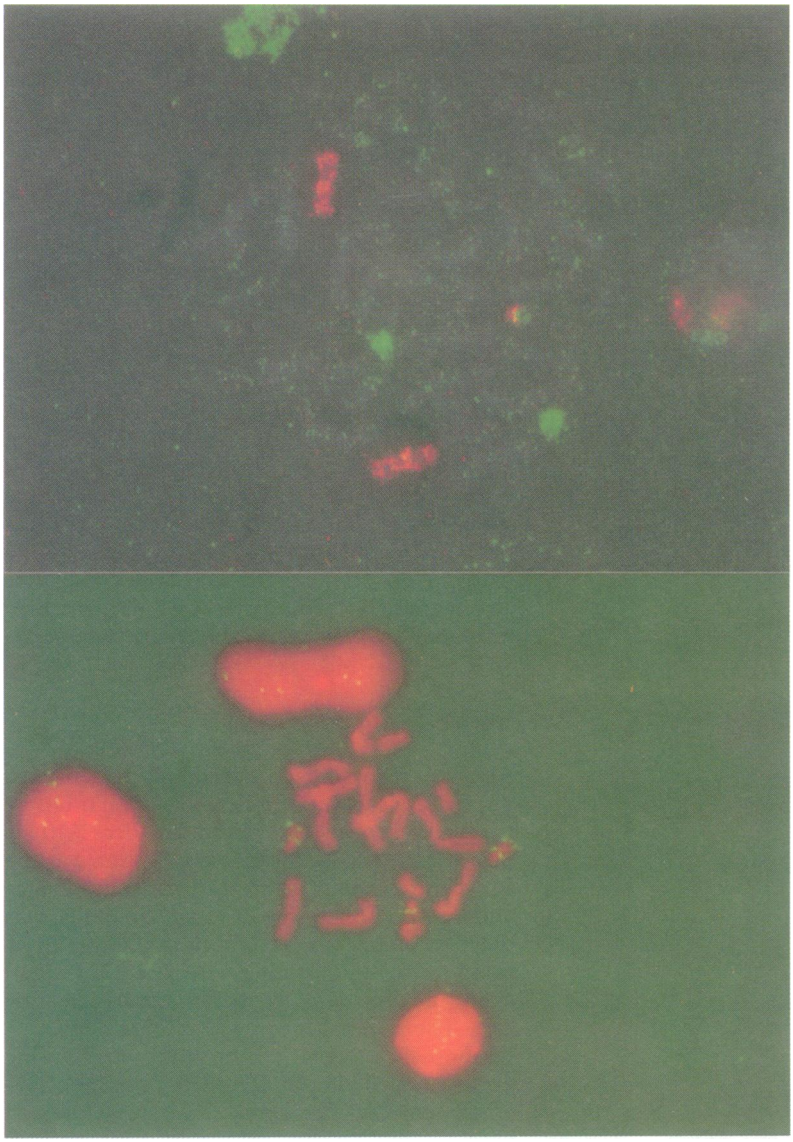

Figure 3 FISH analysis of a metaphase spread of the proband. (Top) Chromosome painting of chromosomes 11 and 22. Chromosome 11 stains red with the rhodamine chromosome 11 painting probe and chromosome 22 stains green with the fluorescein chromosome 22 painting probe. The der(22) chromosome stains green at the pericentromeric end and red at the distal end. (Bottom) The DiGeorge chromosome region probe (D22S75) and the distal chromosome 22 control probe (D22S39) show distinct signals on the normal chromosomes 22 (right and left). The der(22) (centre) shows a signal only with the DiGeorge region probe (D22S75).

mosome painting. Metaphase spreads were probed with digoxigenin labelled chromosome 11 and biotin labelled chromosome 22 painting probes and detected with rhodamine (red signal) and fluorescein (green signal) respectively. The chromosomes were counterstained with DAPI (diaminophenylimidazole). The supernumary chromosome showed green staining of the proximal portion and red staining of the distal portion, confirming its origin from the pericentromeric region of chromosome 22q and the distal region of chromosome 11q (fig 3, top). FISH analysis was also performed with the DiGeorge chromosome region probe, consisting of D22S75, which maps to pericentromeric 22q, and the chromosome 22 control probe (D22S39), which maps to telomeric 22q. The patient's normal chromosomes 22 showed two signals, pericentromeric and telomeric, whereas the der(22) showed a signal only with the pericentromeric 22q DiGeorge chromosome region probe (fig 3, bottom).

RFLP ANALYSIS

Distal locus D22S94 showed that the two different alleles of the proband, 5.5 and 5.4 kb, represent the two maternal homologues (fig 4). The father is homozygous for an allele of 6.6 kb that is not present in the proband. D22S45 also indicates maternal origin of both the proband's alleles. The mother and father are homozygous for the 2.6 and 2.7 kb alleles respectively and the proband is homozygous for the maternal 2.6 allele (data not shown).

Proximal locus D22S43 showed that the proband possesses three alleles of 2.9, 3.8, and 4.8 kb (fig 4). The mother contributed both her 2.9 and 4.8 kb alleles, confirming the inheritance by the proband of both maternal chromosome 22 homologues. The father contributed the 3.8 kb allele. Locus D22S43 maps to the cat eye syndrome region[13] and is located proximal to the t(11;22)(q23.3;q11.2) breakpoint. Thus, the presence of a paternal allele in the proband is consistent with the der(22) being of paternal origin, while the two normal chromosomes 22 are of maternal origin.

PATERNITY

The segregation of 10 microsatellite markers was analysed in order to rule out non-paternity. Analysis was consistent with paternity as stated in all informative cases (data not shown). Based on the results for the six informative loci (* in table 1), the minimal probability of paternity as stated is greater than 99.94%, using only those markers in which the allele contributed by the father was different from that contributed by the mother. Paternity as stated was also consistent for the three VNTR probes. For instance, locus D2S44 shows that the mother had 4.2 and 2.7 kb alleles. The father had alleles of 4.4 and 3.1 kb. The proband had alleles of 4.4 and 4.2 kb, indicating inheritance from both parents as stated (fig 5).

Discussion

The t(11;22)(q23.3;q11.2) is the most frequently identified familial reciprocal translocation in humans.[1–3] This translocation has a low rate of mutation as evidenced by the lack of de novo cases.[3] The only viable, unbalanced karyotype which can result from this translocation is tertiary trisomy for the der(22)t(11;22)(q23.3;q11.2).[1–3] The minimal overall risk for an unbalanced karyotype resulting from this translocation is estimated to be 2%.[3] There is no difference in the recurrence risk between female and male heterozygotes. There is, however, a significant excess of balanced female carriers and a corresponding lack of balanced males among the phenotypically normal offspring of female heterozygotes. This trend is not present among the offspring of male heterozygotes.[3]

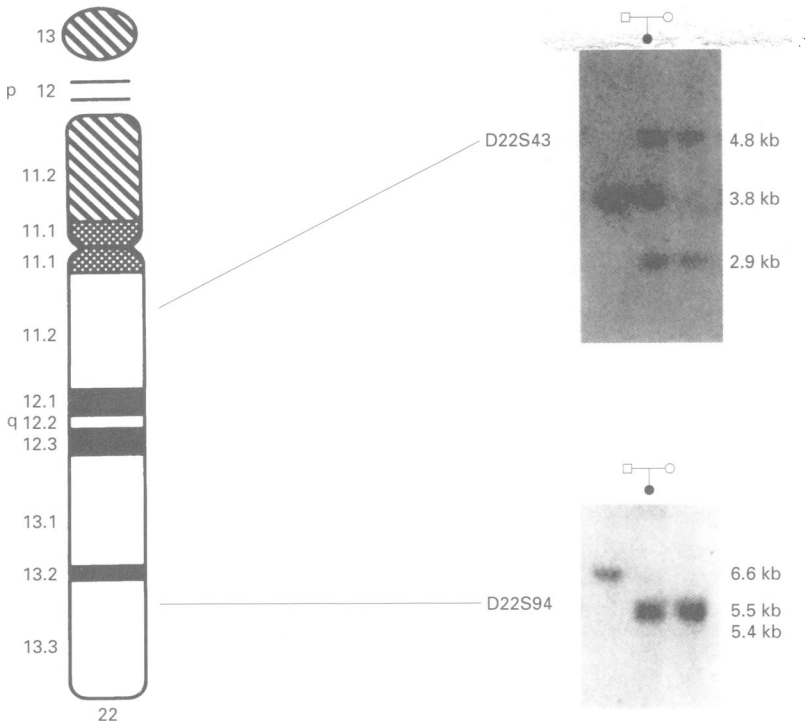

Figure 4 RFLP analysis of the proband and her parents. An ideogram of chromosome 22 indicates the location of the probes used. The autoradiograph of probes D22S43 and D22S55 are shown on the right. Results indicate that the proband inherited two normal chromosome 22 maternal homologues and the der(22) from the father.

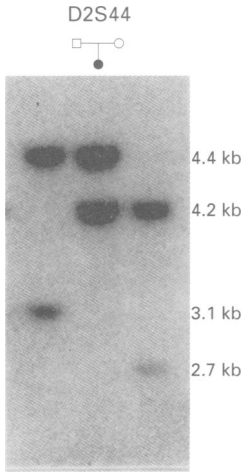

Figure 5 VNTR analysis of the proband and her parents with probe D2S44. The proband inherited the 4.4 kb allele paternally and the 4.2 kb allele maternally. This is consistent with paternity as stated.

The majority of der(22)t(11;22)(q23.3;q11.2) syndrome cases are the result of 3:1 segregation. However, the molecular evidence in the present case suggests that a much more complicated series of events took place. The maternal contribution resulted from meiotic non-disjunction of the normal chromosomes 22, producing a gamete disomic for chromosome 22. The paternal contribution resulted from a de novo t(11;22)(q23.3;q11.2), followed by adjacent 1 segregation, resulting in a gamete which had a normal chromosome 11 and the der(22) but was nullisomic for the normal chromosome 22. Each of these gametes would have normally been non-viable, but through gamete complementation an abnormal offspring resulted with the typical der(22)t(11;22) syndrome.

The meiotic state of non-disjunction is normally inferred from the state of centromeric markers, which are heterozygous in the parent of origin.[19] Locus D22S43 is estimated to be 1–2 Mb from the centromere.[20] In the human genome, 1 Mb of DNA represents approximately 1 cM or 1% recombination.[21] The likelihood of a crossover between the centromere and locus D22S43 resulting in isodisomy for the centromeric region (indicative of an MII error) is very low. The most likely explanation for the maternal heterodisomy of locus D22S43 is a maternal meiosis I non-disjunction event. Loci D22S94 and D22S45, which also indicate maternal heterodisomy, are estimated to be within 5 Mb of the 22q telomere (H E McDermid, unpublished results).

The occurrence of a de novo balanced carrier for the t(11;22) is rare, but has been reported in at least three families.[12] To the best of our knowledge, this is the first case of a de novo der(22)t(11;22) syndrome. The cytogenetic and molecular analysis indicates that the der(22) originated in the germline of the chromosomally normal father.

The proband has maternal heterodisomy for chromosome 22. There have been three cases of maternal transmission of a Robertsonian t(22q;22q) chromosome creating maternal heterodisomy in the offspring.[22–24] In all three cases, the offspring was phenotypically normal. The lack of any phenotypic features to distinguish this case from other der(22)t(11;22) syndrome cases supports the evidence of a lack of imprinting on chromosome 22.

To the best of our knowledge, this is the first reported case of a patient with der(22)t(11;22) syndrome resulting from such a complicated series of genetic errors. However, cases of this syndrome have not been thoroughly investigated for the parental origin of the normal chromosomes 22 and the der(22). This case was analysed molecularly because of the unusual de novo occurrence. It would be of interest to determine how often the standard 3:1 non-disjunction in the carrier parent is not the sole mechanism by which this syndrome results.

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