Trisomy X in a female member of a family with X linked severe combined immunodeficiency: implications for carrier diagnosis

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Abstract

We describe a family affected by X linked severe combined immunodeficiency (SCIDX1) in which genetic prediction of carrier status was made using X chromosome inactivation studies together with limited genetic linkage analysis. Linkage studies in this family showed a confusing pattern of inheritance for the X chromosome. A female with a random pattern of X chromosome inactivation in her T cells appeared to have inherited an X chromosome with four recombinations within 10 cM. The odds of this happening in a single meiotic event make this an unlikely explanation. Data obtained from studying the X chromosomes of her two unaffected sons showed that this could be explained simply on the basis of her having inherited three alleles each of the relevant polymorphic DNA loci. We used fluorescent in situ hybridisation (FISH) to confirm that this person had inherited three complete X chromosomes. Thus, although the results from X chromosome inactivation analysis indicated that this subject was not a carrier of the affected chromosome, FISH and genetic linkage analysis showed clearly that the affected chromosome had been inherited. The implications of this finding for diagnosis of carrier status in this family and for other families with X linked inherited immunodeficiencies is discussed.

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X linked severe combined immunodeficiency (SCIDX1) is a disorder which manifests itself clinically as a loss of cellular, and often also humoral, immunity, owing to a lack of peripheral T cells. SCIDX1 patients therefore suffer from frequent infections, which are fatal by 1 to 2 years of age unless successfully treated by bone marrow transplantation. Non-manifesting women who are at risk of carrying a defective copy of the gene for SCIDX1 can be ascertained by determining a non-random pattern of X chromosome inactivation in their T cells.¹² Genetic linkage analysis is used to support the diagnosis of carrier status from X inactivation patterns, or to indicate carrier status where fresh T cell samples cannot be obtained from the females concerned.

Until recently, the gene for SCIDX1 had been mapped crudely to a region in Xq13-q21.1

between the DNA markers DXS132 and DXS447.³⁴ Further linkage studies have now refined this location to between DXS135 and DXS227 in Xq13.1⁵⁶ The gene encoding the gamma chain of the interleukin-2 receptor (IL2RG) has also been mapped to this region,⁷ and was found to be mutated in 12 separate SCIDX1 families⁷⁻⁹ indicating that mutations in this gene are responsible for SCIDX1.

We have been involved in studies to map new polymorphic markers in this region in several SCIDX1 pedigrees to enable more accurate genetic prediction of carrier status and prenatal diagnosis. Analysis of one family showed a confusing inheritance of alleles which could be interpreted as either the occurrence of a relatively large number of recombinations in two separate meiotic events, or of three copies of the alleles being inherited by one person. We have used fluorescent in situ hybridisation analysis (FISH) to determine which pattern of inheritance is correct.

Materials and methods

PROBES

Restriction fragment length polymorphisms at various loci (DXS159, DXS106, DXS132, DXS441, DXS347, PGK1, and DXS447¹⁰) were detected by Southern blot analysis of genomic DNA, using the specific probes and conditions described previously.¹¹ In addition, dinucleotide repeat polymorphisms at DXS441¹² and PY2-31,¹³ close to PGK1, were amplified as described, but with slight variations: (³²P)-dCTP was included in the reaction mixture rather than an end labelled primer, and 30 amplification cycles of 94°C for 30 seconds, 50°C (PY2-31) or 60°C (DXS441) for 60 seconds, 72°C for 30 seconds, were used. Following amplification, alleles were separated on 6% denaturing polyacrylamide gels and detected by autoradiography. The SSCP in intron 1 of the IL2RG gene⁷ was amplified as described above but with a 55°C annealing temperature. Following amplification, the intron 1 product was digested with 10 units of *Bgl*II for one hour at 37°C; 10 µl of the digested PCR product was analysed on a 1.5% agarose gel, and samples diluted between two and nine times in 10 mmol/l EDTA, 0.1% SDS dependent on the efficiency of amplification. An equal volume of a solution containing 95% deionised formamide, 20 mmol/l EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol was then added and samples denatured at 95°C for three minutes and plunged into

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ice before 4 µl was loaded onto a 6% non-denaturing polyacrylamide gel containing 10% glycerol, alongside an undenatured control sample. Gels were run at 4°C at 60 W for at least 5.5 hours, and alleles visualised by autoradiography. The dinucleotide repeat at the DXS995 locus was used as described.¹⁴ The genetic and physical order of these markers, which extend over approximately 16 cM,¹⁵ is as follows: DXS159-DXS-

106-DXS132-IL2RG-(DXS347, (DXS441-PGK1))-DXS447-DXS995.^{5 6 14 16} The correct assignment of paternity was checked by hybridisation of *HinfI* digested DNA using the hypervariable markers D7S21, D7S22,¹⁷ and D15S86.¹⁸

FLUORESCENT IN SITU HYBRIDISATION

The methods used for both interphase and metaphase FISH were essentially as described by Katz *et al.*,¹⁹ with slight modifications as summarised below.

Metaphase FISH

Mitoses were obtained for analysis from cryo-preserved peripheral blood mononuclear cells, cultured for three days before analysis in 1 ml of RPMI 1640 (Life Technologies Ltd, UK) supplemented with 1 mmol/l L-glutamine (Life Technologies Ltd, UK), 20% FCS (Globe-pharm Ltd, UK), 50 µg/ml gentamycin (Life Technologies Ltd, UK) 25 units/ml human recombinant interleukin-2, and 60 ng/ml phytohaemagglutinin (Murex Diagnostics, UK) at 37°C in 5% CO₂. The cells were harvested and G banded using standard procedures. Duplicate slides were then denatured and dehydrated before being incubated overnight at 37°C with 15 µl of a biotinylated X specific whole chromosome paint (Cambio, UK).

Interphase FISH

Cryopreserved peripheral blood mononuclear cells were thawed rapidly, washed twice with PBS, resuspended at 10⁶ cells/ml in methanol: acetic acid 3:1, and one or two drops of fixed cells placed in the centre of clean, dry, glass slides. Once dry, slides were dehydrated, then denatured in 70% formamide/ 2 × SSC for two minutes at 70°C before dehydrating again; 10 ng each of biotinylated chromosome X specific and digoxigenin labelled chromosome 7 specific α satellite probes (Oncor, Gaithersburg, USA) were combined and diluted into the hybridisation mixture, denatured, preannealed, and then hybridised to the slides overnight.

Signal detection

Following hybridisation, slides were washed and the signal detected using 5 µg/ml avidin-FITC (Vector Labs, UK) for detection of the biotinylated X chromosome specific probes, and 5 µg/ml anti-digoxigenin-RITC (Boehringer, UK) for detection of the digoxigenin labelled chromosome 7 specific probe. Incubations were performed in the dark. Following signal amplification with biotinylated anti-avidin (Vector Labs, UK), slides were mounted in antifade solution (Citifluor Ltd) containing 2 µg/ml of the counterstain DAPI (Sigma, UK) and examined using a Zeiss Axioskop microscope equipped with a CCD camera (Photometrics, UK) connected to a Macintosh Quadra computer. Image analysis was performed using an IP Lab (UK) spectrum package.

Figure 1 (A) pedigree of the family showing individual haplotypes at eight polymorphic marker loci. Black dot indicates an obligate carrier; NR= unilateral pattern of X inactivation; R= random pattern of X inactivation. Black squares represent affected males. Numbers 1 and 2 differentiate the SSCP alleles, and indicate, respectively, presence or absence of an RFLP site. Alleles of decreasing C_An repeat length are distinguished by numbers from 1-8. Arrows indicate minimum region in which a recombination is presumed to have taken place. (B) Inheritance of alleles at the SSCP within intron 1 of IL2RG, the results of which indicate that II-4 has not inherited the affected allele. Lane 1 is undenatured normal DNA. Lanes 2-7 are denatured samples, loaded according to the partial pedigree shown above the gel. Single strands corresponding to alleles 1 and 2 are labelled to the left of the figure. DS is double stranded DNA.

Figure 2 Pedigree of the family showing the inheritance of trisomy X. Symbols and numbers are described in the legend to fig 1A.

Figure 3 Inheritance of alleles at DXS995. A partial pedigree is shown above the gel, corresponding to the loading of lanes 1-7. The alleles are labelled to the left of the figure. Although a number of "shadow" bands are present, it is apparent that II·4 has inherited all three alleles (alleles 1 and 2 can be distinguished on the basis of inheritance by her two sons).

Results

The results of linkage studies in this family are shown in fig 1A. A recombination between the markers DXS159 and DXS106 in subject II·4, a female with no affected sons, was described previously.²⁰ X chromosome inactivation studies in the T cells of this person suggested that she was unlikely to be a carrier of SCIDX1 since the inactivation pattern appeared to be random.²⁰ Further linkage studies showed a second recombination in the same chromosome, between the markers DXS347 and PGK1, suggesting that subject II·4 had inherited the carrier chromosome in the region of the IL2RG gene. However, analysis with the SSCP in intron 1 of IL2RG showed that the normal copy of this gene had been inherited (fig 1B), suggesting a further two recombinations in this chromosome. Results using the hypervariable markers D7S21, D7S22, and D15S86 indicated that paternity was likely to

Figure 4 FISH analysis of a metaphase spread from a T cell of subject II·4, showing the presence of three complete X chromosomes ("painted" white).

be as assigned, and that the DNA from subject II·4 had not been contaminated by DNA from other members of the family (results not shown).

We then studied the two unaffected sons of subject II·4 and showed that one, subject III·2, had inherited the grandpaternal chromosome. However, the second son (subject III·3) appeared to have inherited a chromosome with four recombinations (fig 1A). According to Drayna and White,²¹ between one and four recombinations would be expected on the X chromosome in each meiosis. However, in this family, four recombinations appear to have occurred within only a very small region of the X chromosome in two separate meioses. Since the probability of this occurring is therefore very low, we looked for a simpler explanation for the inheritance of alleles in this family. Since subject III·3 had inherited the same chromosome as his uncle (II·7) it seemed likely that his mother (II·4) had also inherited this chromosome. The confusing pattern of alleles in subject II·4 could therefore be because of the presence of a third chromosome as a result of a non-disjunction event in the gamete from I·2 (fig 2).

In order to test this theory, we first used the hypervariable microsatellites DXS986, DXS995, and DXS1002 to try to identify all three chromosomes inherited by subject II·4. Only DXS995 was found to have different alleles for the three chromosomes, and subject II·4 was found to have inherited all three of these alleles (fig 3). We then used FISH analysis on interphase lymphocytes from subject II·4 using α satellite chromosome specific probes for chromosomes X and 7 (as a control). This showed that subject II·4 had three copies of the X chromosome specific probe in her cells, as opposed to only two copies of the chromosome 7 specific probe as expected (data not shown). Finally we repeated FISH on dividing T cells using an X chromosome specific paint, and showed the presence of 47 chromosomes, with three complete X chromosomes, in 10 out of 10 separate metaphases from the cells of subject II·4 (fig 4).

Discussion

A non-manifesting female in a SCIDX1 pedigree was diagnosed as not carrying the mutated allele on the basis of a random pattern of X inactivation in her T cells. We have now shown that this woman has inherited three complete X chromosomes, one of which is the chromosome carrying the SCIDX1 mutation. Since this X chromosome will appear inactivated in all her T cells, the random X inactivation pattern has arisen as a result of her random inactivation of the other two non-mutant X chromosomes. This woman is therefore a carrier of SCIDX1 despite previous evidence to the contrary.

Trisomy X is reported to occur at a frequency of about 1 in 1000 women and, although intelligence tends to be below average, and approximately 25% of such women are infertile, there are no obvious abnormalities.^{22,23} There is therefore a low risk for women in pedigrees affected by other X linked disorders having their carrier status misdiagnosed owing to this phenomenon. For example, carriers of other X linked immunodeficiencies, such as agammaglobulinaemia (XLA) and Wiskott-Aldrich syndrome (WAS), have also been determined by detection of non-random X chromosome inactivation patterns in purified B cells or whole blood, respectively.²⁴⁻²⁷

Failure to identify events such as trisomy X in genetic linkage studies of X linked disorders could lead to the incorrect interpretation of marker genotypes and hence incorrect localization of the gene. Partial chromosomal duplications can have a similar effect, as shown by studies of Charcot-Marie-Tooth disease type 1A.²⁸ The use of more sensitive hypervariable markers for linkage analysis should enable detection of such events as trisomy X if these markers are informative for all three chromosomes. In addition, the cloning of the genes involved in such disorders will enable unambiguous assignment of carrier status even in such cases of unusual inheritance. SCIDX1, for example, is caused by mutations in IL2RG,^{7,8} and mutations in a tyrosine kinase gene (*Btk*) have recently been shown to cause XLA,²⁹⁻³¹ enabling unambiguous assignment of carrier status and prenatal diagnosis in families affected by these disorders.³²

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