Deletion mapping of the DXS986, DXS995, and DXS1002 loci defines their order within Xq21

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Abstract

The three microsatellite repeat loci, DXS986, DXS995, and DXS1002, have been mapped to Xq13.2-21.1. We report here their relative order and their localisation within Xq21. These loci will be useful for the genetic mapping of disease loci in this region, in particular X linked deafness, as DXS995 lies in the region critical for this disorder.

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A number of genetic disorders have been mapped to the Xq13-21 region, including X linked severe combined immunodeficiency (SCIDX1),¹ X linked deafness,² and a range of mental retardation syndromes,³ by genetic linkage studies. Such studies are dependent on the use of well mapped, highly polymorphic markers such as microsatellite repeats. DXS986, DXS995, and DXS1002 are loci detectable by microsatellite repeat markers with polymorphism information content (PIC) values of 0.76, 0.61, and 0.72, respectively.⁴ These have been mapped crudely to Xq13.2-21.1 by genetic linkage analysis of CEPH families.⁴

To map these markers with respect to one another and to refine their positions, we have used genomic DNA from three patients with interstitial deletions on the proximal long arm of the X chromosome. Patient NP has a deletion which extends from the DXS447 to DXS73 loci (Xq21.1-21.33) and results in choroideremia, mental retardation, and cleft lip and palate but no immunodeficiency.² The proximal breakpoint of this deletion defines the distal end of the SCIDX1 critical region.⁵ By contrast, patient MBU is deleted from DXS233 to pF1 (Xq21.1-22) and has only choroideremia, whereas patient D20 is deleted from DXS232 to DXS73 (Xq21.1-21.33) and has choroideremia, mental retardation, and deafness.²

Genomic DNA was prepared from EDTA blood samples⁶ (normal male and patient D20) and EBV transformed B cell lines⁷ (patients NP and MBU). Oligonucleotide primers specific for the DXS441 locus⁸ and DXS986 (AFM116xgla 5'-CCTAAGTGCTCATCAT-CCCA-3' and AFM116xglm 5'-AGCTCAATC-CAAGTTGCTGA-3'), DXS995 (AFM207zg5a 5'-AAGGGGCTGCTGATGATTAT-3' and AFM207zg5 m 5'-AATGCGTTCCCCAAA-TGT-3') and DXS1002 (AFM249vh5a 5'-CTGCTACCCTTTAGTTCTCTC-3' and

AFM249vh5m 5'-TCCATGTTGCTGCG-AA-3') were prepared and used for PCR amplification. A reaction volume of 25 µl was used with 100 ng of genomic DNA and 10 pmol of each primer in 10 mmol/l Tris-HCl, pH 8.3, 50 mmol/l KCl, 1.5 mmol/l MgCl₂, 200 µmol/l dATP, dGTP, dTTP, and dCTP, and 1 unit of *Taq* polymerase (Bioline). Thermal cycling started with denaturation at 94°C for three minutes, followed by 30 cycles at 55°C (DXS441 and DXS1002) or 60°C (DXS986 and DXS995) for one minute/72°C for 30 seconds/94°C for 30 seconds, and finally a 10 minute extension step at 72°C. DNA from a normal male and the amplification of DXS441 were used as positive controls; 10 µl of the reaction was analysed on a 2% agarose gel.

The breakpoints in the deletion patient DNA divide the Xq21.1-21.33 region into four intervals as shown in the figure. The results of PCR analysis of patient DNA samples using the primers specific for the DXS441, DXS986, DXS995, and DXS1002 loci are also shown in the figure. Considering the results obtained for DXS986, the primers amplify a product in MBU and D20 but not NP DNA; therefore this locus must lie in the interval between the proximal breakpoints of NP and D20. Similarly, based on the results obtained for DXS995 and DXS1002 with the same DNA samples, these loci can be assigned to two of the other three intervals as shown in the figure. Thus, DXS986 must lie between PGK1 and DXS232, DXS995 between DXS26 and DXS233, and DXS1002 between DXS121 and DXS96. Since all these loci are within the NP deletion they must all be distal to Xq13.3. This is confirmed by the fact that none of these loci was present within YAC clones forming a contig in the Xq13.3 region (data not shown^{10,11}). The order of loci in the Xq13.2-21.33 region can therefore be deduced as: cen-PGK1-[DXS986, (DXS447-DXS72-DXS26)]-[DXS995, (DXS232-DXS121)]-[DXS1002, (DXS233-DXS1-DXS3-DXS73)]-DXS96-pF1-tel.

The SCIDX1 gene has recently been identified as the interleukin-2 receptor gamma chain (IL2RG) which maps to Xq13.1.^{12,13} Our data confirm that all three of these loci are distal to the SCIDX1 locus. As these microsatellite repeat markers have a high PIC value, their more precise localisation will be useful for linkage mapping, carrier determination, and prenatal diagnosis of other disorders which map to this region. In particular, DXS995 may be useful with respect to X linked deafness as it

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Results of mapping the microsatellite repeat loci DXS986, DXS995, and DXS1002 in DNA from patients MBU, D20, and NP and from a normal male. Idiogrammatic representations of the interstitial deletions in the long arm of the X chromosome of these patients are shown in the top half of the figure. Loci mapped to specific intervals are given in their relative order on the right^{5,9} and localisation of the microsatellite repeat loci on the left. In the lower half of the figure are shown the results of amplification with primers specific for the microsatellite repeats in the DNA (lanes 2 to 5); 1 kb ladder DNA was used as a size marker (lane 1) and a few of the fragment sizes (bp) are shown on the left. The size ranges of the amplification products are as follows: DXS441 173–189 bp; DXS986 149–173 bp; DXS995 193–199 bp; DXS1002 266–274 bp.

lies in the region critical for this disorder, between DXS26 and DXS121, as defined by deletion mapping.² Following extensive mapping studies in the Xq12–21.3 region, including this work, microsatellite loci have now been located at approximately 2 to 3 Mb intervals throughout this region¹⁴ which will aid further gene mapping studies.

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