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The Characterisation of Human X-linked Polymorphic Markers and Their Use in Disease Gene Localisation and Identification

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Summary

At the start of this project a number of groups were beginning to develop chromosome specific microsatellite based genetic linkage maps. In this project AC dinucleotide repeat markers were isolated from a bacteriophage library for application to the genetic localisations of X-linked disease genes, particularly those responsible for non-specific mental retardation (*MRX*). Of eleven novel microsatellite markers isolated and characterised (Chapter 4), seven were genotyped through the CEPH families enabling their incorporation into a PCR-based linkage map of the X chromosome. In addition, six other pre-existing microsatellite markers were also genotyped through the CEPH families, and included in the map (Chapter 5). The map consists of seventy-eight markers, sixty-nine of which are PCR-based with an average heterozygosity of 66%. The map spans 236 centimorgans, from the *XG* locus near Xpter to *DXS52* near Xqter, and the average spacing between the seventy-eight markers is 3.1 cM.

This map was then used to refine the localisation of the disease gene segregating in five families affected with X-linked mental retardation (Chapter 6). Three of these families (*MRX19*, *MRX30* and *MRX31*) had males with non-specific mental retardation and the other two families (W and O'H) had males with additional symptoms. The *MRX19* gene has been localised to Xp22, within a 19 cM region between the loci *DXS1043* and *DXS1052*. The *MRX30* gene has been localised to a 28 cM region between the loci *DXS990* (Xq21.33) and *DXS424* (Xq23). The *MRX31* gene has been localised to a 12 cM region between the loci *DXS1126* (Xp11.23) and *DXS1124* (Xq13). These results enabled carrier risk analyses to be offered to the females of these three families. Linkage analysis of family W excluded the gene from most of the long arm of the X chromosome, but revealed significant lod scores to two large regions mainly confined to the short arm, in which the disease gene may lie. The first region is from Xqter to *DXS1068* (Xp11.4) and the second region lies between *MAOA* (Xp11.4) and *DXS1002* (Xq13.3-q21.1). The linkage analysis of family O'H has shown significant lod scores to two relatively small regions: the disease gene lies either between *DXS1275* (Xq12) and *DXS7113* (Xq12-q13.1) or in Xq23 between *DXS1220* and *DXS8067*.

A large proportion of those families affected with non-specific X-linked mental retardation have had the causative gene localised to regions that span the centromere. The isolation and characterisation of genes, with expression in the brain, near this region

would therefore generate candidate genes for these MRX loci. To this end, YACs were identified using primers from one microsatellite marker (*DXS1125*) which mapped to this region. From these YACs, *Alu*-PCR products were generated to identify cosmids from which an attempt was made to isolate exons. Five putative exons were trapped (Chapter 7). This approach was abandoned, however, because the trapped sequences were not exons, and because of the recent generation of thousands of expressed sequence tags *via* single-pass sequencing of cDNA by other investigators. Instead, two candidate genes (*CR73E* and *DXS6673E*) were screened for mutations, by SSCA, using the DNA from members of several families with X-linked mental retardation whose disease gene localisations overlapped the positions of these two genes (Chapter 7). Two polymorphisms were discovered in the *CR73E* gene that enabled it to be eliminated as a candidate for the *MRX12* family.

The genetic map described in Chapter 5 was also used in the linkage analysis of a family with males affected with spastic paraplegia (Chapter 8). The results of this analysis led to an investigation of a candidate gene: *PLP*. A point mutation, $T^{707}C$, was found in exon 6 of this gene that would lead to a Phe^{236} to Ser amino acid substitution. A sporadic case of a boy affected with myotubular myopathy was also investigated. The hypothesis was made that the disease in this boy was X-linked and this led to the screening of the *MTM1* gene for any mutations. A $C^{775}T$ mutation was discovered in exon b. This would lead to a Arg^{259} to Cys amino acid substitution. A third family was also investigated. This family had males affected with Wiskott-Aldrich syndrome. Mutation screening of the *WAS* gene identified a $G^{407}A$ transition in exon 4. This would lead to a Gly^{125} to Arg amino acid change. In all three cases, evidence is provided that strongly suggests the mutations found can be held responsible for each disease phenotype, and as a consequence, direct tests are now available to the members of these families. In addition, the significance of genotype in relation to phenotype may prove useful in the prognosis of affected males from other families with these diseases.

An alternative to the approach of finding disease genes as given in Chapter 7, is to investigate the molecular basis of cytogenetic abnormalities. Chapter 9 describes the preliminary physical mapping of two translocation breakpoints and one duplication event. The first translocation occurs in Xp22 and may be responsible for the global

developmental delay experienced by the female carrier. The second translocation occurs in Xq24 in a female suffering from manic depression. This region has also been implicated, by linkage analysis, in the manic depression segregating within a Finnish family. A third female is the mother of a boy who had symptoms of Borjeson-Forssman-Lehmann syndrome. She carries the same duplication as her son. The point at which the duplication begins occurs in Xq26 and this region is included in the localisation of this disease gene from linkage analysis of a family with this syndrome. YACs have been selected from published and unpublished physical maps and used for fluorescence *in situ* hybridisation to progressively narrow the regions within which these two translocations and the point of duplication have occured. This work provides the basis for the cloning of any genes involved in the manifestation of each of these three X-linked disorders.