MAPPING GENES FOR X-LINKED DISORDERS

by

Ágí Kyra Gedeon B.Sc.(Hons.)

This thesis is submitted as the complete requirement for the degree of Doctor of Philosophy within the Department of Genetics, Faculty of Science, at The University of Adelaide, South Australia.

September 1996

Table of Contents

Summary

The human genome project aims to sequence the genome, identify genes and thus the causes of inherited disease. The research presented in this thesis reports contributions to the development of the evolving map of the X chromosome, and the localisation and genetic delineation of rare X-linked monogenic neuropsychiatric diseases and other X-linked disorders, culminating in the identification of two new genes.

New polymorphic PCR-based markers were characterised from probes of known location (DXS102, DXS237, DXS294 and DXS300). Three were physically mapped between somatic cell hybrid breakpoints and on a YAC contig of Xq25-27. Development of a regional genetic map contributed to the integration of maps in Xq26. 40 CEPH reference families were genotyped for these markers and another, DXS538, and added to a comprehensive genetic map constructed from the CEPH database (ver 5.0). Additional PCR-based markers gathered from the literature, were ordered by comparisons with other genetic and physical maps, to create a composite background map spanning the entire X chromosome to facilitate localisation of disease genes by linkage.

The disproportionate excess of retarded males in the population may be accounted for by a number of X-linked disorders with mental retardation as a feature. The fragile X syndrome is responsible for less than half of these. To determine the number and distribution of other genes involved in XLMR, fifteen non-FRAXA families segregating mental retardation were collected into two distinct classes: i) MRX, where the mental retardation was the sole and major feature in otherwise clinically apparently normal individuals and ii) MRXS, with associated clinically distinct features. The disease gene in each family was localised by demonstration of significant linkage to markers in an interval flanked by recombinant events. These linkage studies identified 7 distinct regions of the X chromosome involved in MRX and confirmed the genetic heterogeneity of MRX.

The genes for five clinically distinct mental retardation syndromes (PRTS, BFLS, WTS, SHS and XLMR with macrocephaly) were similarly localised or their localisations refined, but overlapping localisations with MRX genes cannot separate these entities from allelic mutations in the same genes. Two candidate loci, identified by position, were analysed by single stranded conformation analysis (SSCA) and sequencing to detect mutation. One at SOX3 in the BFLS region did not yield any differences between normals and BFLS affecteds, the other at the DMD brain promoter detected a C to T base substitution in a normal male from an MRX family and does not appear to have a deleterious effect within this highly conserved promoter.

Other genes for X-linked disorders were genetically localised using the assembled resources and the linkage approach. New localisations include the genes for a pigmentary disorder with systemic manifestations (PDR) previously known as X-linked cutaneous amyloidosis, a Lutheran blood group suppressor (XS) and two forms of cardiomyopathy; Barth syndrome (BTHS) and the possibly allelic form of fatal infantile cardiomyopathy. Localisation of BTHS has lead to collaboration resulting in the identification of the BTHS gene (G4.5) by the positional candidate approach. The disease-causing mutation has been identified in the BTHS family and work is under way to investigate allelism of fatal infantile cardiomyopathy.

Males with submicroscopic deletions of the X chromosome can be valuable for recognising and isolating disease genes, as they identify all the phenotypic associations with the deleted gene(s). Deletion of the FMR1 gene and adjacent sequences was associated with only the characteristic fragile X phenotype in one boy. This was the first case described with fragile X syndrome due to deletion of FMR1 and supported the hypothesis that FMR1 was indeed the gene solely involved in the fragile X syndrome. Deletions detected in Xq28, over 600kb distal to FMR1, were associated with global developmental delay in one boy and with speech delay in another. Characterisation of these deletions and demonstration that the deleted sequence was conserved in evolution, lead to the isolation of the FMR2 gene at FRAXE. This gene is the eighth locus responsible for non-specific X-linked mental retardation (MRX) identified in this study. Others have identified further MRX loci in contiguous gene syndromes at Xp22.3 and Xq21 and a translocation breakpoint in Xq13 to physically discrete intervals. Up to 11 distinct regions/genes causing X-linked mental retardations (including FMR1) have now been established confirming the genetic heterogeneity of XLMR.

These studies have focused on gene localisation in X-linked disorders, particularly those with mental retardation. Genetic risk estimation has been made available to family members when required. A significant contribution has been made to the gene map of the X chromosome and to the delineation of genes responsible for XLMR. At least 10 discrete MRX genes, distinct from FMR1 and each other, account for a proportion of the excess of non-FRAXA retardation in the male population. The regional localisations described upon the background map of the X chromosome, include ten loci for MRX, five syndromal forms of XLMR and four other X-linked conditions. The physical characterisation of the deletion in each of three boys and maintenance of fruitful international collaborations have made possible the identification and eventual cloning of the disease genes involved in two of the clinical conditions studied and have contributed to the mapping of the X chromosome.