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Identification of Novel Genes for X-linked Mental Retardation

A thesis submitted for the degree of Doctor of Philosophy to the University of Adelaide

by

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SUMMARY

Mental retardation (MR) is estimated to affect 1-2% of the population, and is due to both environment and improperly functioning genes. A higher incidence of MR in males suggests a significant proportion of MR with a genetic cause is due to mutations in genes on the X-chromosome. The aim of this thesis is to identify novel genes involved in X-linked mental retardation (XLMR). Understanding the genetic causes of MR will result in better diagnosis, and as a result, will lead to improved patient management and counselling of family members. It will also contribute to the understanding of the genes and mechanisms required for normal cognitive function.

The first part of the project involved molecular characterisation of the breakpoints of three X chromosome rearrangements in three unrelated patients with MR, to identify candidate genes for familial XLMR.

For Patient 1 an inversion breakpoint was found to lie within the 3' untranslated region (3'UTR) of the biglycan gene (BGN), such that BGN in this patient obtained a new 3'UTR and polyadenylation signal from Xq13.1. The open reading frame of BGN remained intact, and apparently normal levels of mRNA transcribed. 3'UTRs have been shown to contain elements important for mRNA localization, stability and translation efficiency. Therefore, in this patient disruption of elements in the 3'UTR may affect the levels of BGN protein produced and thereby cause MR. Characterisation of this inversion has identified BGN as a candidate gene for XLMR.

For Patient 2, who also had an inversion of the X chromosome, all BAC clones derived from Xq28 that were used as probes for FISH gave spanning signal. This suggested that this patient
had a previously undetected duplication of Xq28 as well as the initially detected inversion. This duplication covers at least 2.7 Mb of Xq28, an extremely gene rich region. Thus the MR in this patient is likely to be the result of functional disomy of many genes from Xq28. This work suggests that some familial cases of XLMR may be due to submicroscopic duplication of many genes, a mechanism that would not be detected by current PCR based methods of gene screening.

For Patient 3 a translocation of the X chromosome associated with MR and cardiomyopathy was characterised. In this patient, the normal X chromosome is preferentially inactivated suggesting that disruption of a gene on the X chromosome may be responsible for the phenotype. The break at Xq28 was located within a 75 kb BAC clone. Although not physically disrupted by the translocation breakpoint one gene, TMG3a, whose expression was affected by the breakpoint was identified. This gene will therefore also be a candidate gene for XLMR as well as for X-linked cardiomyopathy.

The second part of the thesis involved positional candidate gene screening in a family that was localised to two regions of the X chromosome by linkage analysis. Additional family members for a linkage study were gathered and analysed and Xq13 became the most likely localisation. This region was gene rich and contained many good candidate genes for XLMR. A mutation screen of these genes has begun; as yet no disease causing changes have been identified, and this project remains ongoing.

The final part of this project has been the identification of the homeobox gene, ARX, and its role in both syndromic and non-syndromic XLMR. Initially, candidate gene screening within the minimal linkage interval for X-linked infanile spasms syndrome (ISSX) was undertaken,
in order to identify the gene responsible for this disorder, which is characterised by infantile spasms, hypsarhythmia and severe to profound MR. Expansions of two polyalanine tracts in the ARX gene were detected in three families with ISSX, as well as a truncating mutation in a more severe case of ISSX. Subsequent screening of other XLMR families has shown that mutations in ARX account for a significant proportion of NSXLMR as well as other syndromic cases of XLMR. Mutations in ARX account for approximately 8% of MR where there is a clear X-linked inheritance and is now the most significant cause of MR in mapped MRX families.

Polyalanine tract expansions have been detected in other genes. In the case of PABP2, mutations in which cause oculopharyngeal muscular dystrophy, the polyalanine expansion results in the formation of protein aggregates in cells of patients. Transfection of HeLa cells with ARX fused in frame with green fluorescent protein (GFP) has shown that ARX with an expanded polyalanine tract does not form aggregates. These were preliminary studies and further confirmation of this result is required before conclusions can be drawn about the mechanism by which these ARX polyalanine expansion cause MR.

This thesis has therefore addressed the identification of candidate genes for XLMR using two positional cloning approaches. Firstly positional cloning of X chromosome rearrangements in patients with MR, and secondly by positional candidate gene screening of genes within minimal linkage intervals of mapped XLMR in large families. This work has identified two candidate genes BGN and TMG3a, which can now be screened in other XLMR cases. It has also resulted in a refined linkage interval in a large NSXLMR family, in which screening of candidate genes has begun. Finally, mutations in the gene ARX have been identified as a significant contributor to the aetiology of XLMR.