

Analysis of the GPR50 Gene's Involvement in X-Linked
Mental Retardation in Chromosomal Region Xq28

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Introduction

The possibility of X-linkage being involved in mental retardation was first seriously considered in the 1970s after the identification of the Fragile X syndrome as a distinct clinical condition. X-linkage for mental retardation was also supported by earlier evidence with mental retardation in large families showing a X-linked pattern of inheritance on their genetic pedigree and by epidemiological studies showing that approximately 30% more males than females traditionally have mental retardation. Two major classifications of X-Linked Mental Retardation (XLMR) have since been found to exist: syndromic XLMR and non-specific XLMR. In syndromic XLMR, the mental retardation is only one aspect of a distinct clinical set of symptoms such as in Coffin-Lowry and Rett syndromes. In non-specific mental retardation, mental retardation is the only condition present. The causes underlying XLMR are also extremely varied and diverse involving genes in neuronal development, chromatin remodeling, and signal transduction (Chelly and Mandel 2001). This diversity of etiology is one of the primary factors in making XLMR the current “black box” in genetic research (Max Planck Institute for Molecular Genetics).

Aims of Research

My specific research project is on the GPR50 gene located on the q arm of the X chromosome in region 28. The GPR50 gene encodes the H9 G-protein coupled receptor that is related to other high-affinity melatonin receptors but unable to bind to melatonin (Gubitz and Reppert 1999). Melatonin is a hormone of the pineal gland, which regulates circadian rhythms in mammals, and until recently, three main receptor subtypes were known to be expressed in the brain until the identification of H9, a receptor composed of 613 amino acids. The H9 receptor does not bind to either melatonin or to its agonist 2-[¹²⁵I]iodomelatonin.

However, H9 is still expressed in both the pituitary and hypothalamus leading to a probable endocrine or neurological function. The GPR50 gene that encodes H9 appears to be comprised of two exons separated by an intron (Reppert et al. 1996).

Methods

In my particular project, I will be looking for mutations within the GPR50 gene using a gene pool of males with XLMR of unknown cause. I will look for mutations in exon one using a nested PCR amplification technique and dideoxy sequencing. Nested PCR is run the same way as regular PCR by heating to denature the DNA, then cooling to anneal the primers, and heating again to allow the DNA polymerase reaction to occur (Tamarin 1999). The only difference is that it is done twice amplifying first a larger and then a smaller area. After the PCR amplification steps, the DNA would then be sequenced using four subsamples with nucleoside triphosphates (A, T, C, & G), DNA polymerase, and different dideoxy nucleotides to obtain the sequence. This dideoxy sequencing would be interpreted by previous radioactive labeling and autoradiography (Tamarin 1999).

I will look for mutations in exon two in much the same fashion except for two “gap regions.” In gap one, I will use restriction endonuclease fingerprinting or REF. In REF, the PCR products are digested with several overlapping restrictive enzymes, and the digested products are then recombined. The mixture would then be denatured and mutations would be identified using gel electrophoresis. The different nucleotide mutation sequences causing different intrastrand bonding would then lead to different migration patterns in the gel producing the REF. The gel REF would then be made into an autoradiograph for analysis (Du et al. 1998). Gap two mutations will be identified using a DHPLC technique, which could not be used in gap one due to an uneven denaturing temperature. In DHPLC or

denatured high performance liquid chromatography, samples of DNA are injected into a port in which they heated to the point of denaturing at this point the strands reanneal with each other forming three possibilities, if mutants are present. Wild-type could anneal with wild-type, mutant could anneal with wild-type, and mutant could anneal with mutant. These different combinations then provide different sedimentation rates as the DNA in the liquid phase is carried past the stationary phase in the column (Bardwell et al. 2002). If any mutations are then found in either gap one or two, dideoxy sequencing would be then used to determine the nature of the mutation. These experiments are planned for the Fall of 2002 and the Spring 2003.

References

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