GENE MAPPING

MAPPING THE VASOPRESSIN V2 RECEPTOR GENE NEAR NEPHROGENIC DIABETES INSIPIDUS

Stephen T. Warren, PH.D.

From the Howard Hughes Medical Institute and Departments of Biochemistry and Pediatrics, Emory University School of Medicine, Atlanta, GA

Recent reports by Birnbaumer et al.1 and Lolait et al.2 describe the cloning and mapping of the vasopressin V2 receptor to the human chromosomal band Xq28, suggesting this locus to be the gene responsible for nephrogenic diabetes insipidus (NDI). Fittingly, it will be 100 years ago this October that McIlraith3 first described NDI in three generations of males with extreme thirst and deduced the X-linked nature of the inheritance. NDI is characterized by renal tubular insensitivity to antidiuretic hormone (ADH) leading to polydipsia, polyuria, and hypostenuria. The inability to concentrate the urine can lead to severe dehydration, particularly in infants, and has been associated with failure to thrive as well as mental and growth retardation. However, with adequate fluid intake, renal function is normal and the sequelae of this end-organ unresponsiveness to ADH is prevented.

NDI has an interesting historical past, as is sometimes uncovered with genetic disease with clear inheritance and obvious clinical signs. It is believed that most New American families originated from the Ulster Scot clan who landed in Halifax, Nova Scotia, from Ireland in 1761 (also in October) aboard the ship Hopewell. The tales of “water-drinkers” is well documented throughout the province and has been summarized by Bode and Crawford4 in an article well worth an evening’s read. Although the descendants of these colonists have now moved throughout the continent, it is likely, based upon genealogical and historical records, that most NDI patients in North America have common ancestors of Ulster Scotsmen and therefore the “Hopewell hypothesis”5 would predict a common mutation at the DNA level. While the mutation rate at the NDI locus is therefore seemingly low, it is important to note that unlike Huntington’s chorea, where only 1 or perhaps 2 original mutations are believed to have occurred, reports of Somoan and Australian aboriginal cases suggest new mutations are possible, though none have been reported as such.

The gene responsible for NDI has been increasingly well mapped within human Xq28. Linkage analysis6-9 has shown very tight linkage NDI and Xq28 marker loci and recent studies by Van Den Ouweland et al.10 describe recombinant individuals that would place the NDI locus distal to DXS 305. Based upon the physical map of human Xc28, the region distal to DXS 305 is approximately 4.5 Mb in size.11 Finding the NDI gene by positional cloning approaches, while significantly improved in recent years, remains an arduous task through a region of this magnitude. Another course toward the NDI locus, becoming more common with the increasing density of the human gene map, is the candidate gene approach where the colocalization of a disease locus with position of a gene whose function is well enough understood to propose that the two loci are in fact the same. Such is the case with NDI.

The pathophysiology of NDI can be explained completely by the failure of the collecting ducts to increase their water permeability in response to the antidiuretic hormone vasopressin, resulting in the excretion of urine which is hypotonic to plasma.12 It was conclusively demonstrated by Williams and Henry13 that the concentrating defect in NDI was due to end-organ unresponsiveness to vasopressin since patients failed to exhibit a change in urine volume or concentration despite near toxic administration of the analogue pitressin. Since serum vasopressin levels increase normally to increasing plasma osmolality in patients, the urine osmolality remains unchanged,14 suggesting that the defect is in the renal vasopressin receptor, or distal components normally modulated by ligand-receptor binding. The gene encoding the vasopressin V2 receptor (the renal vasopressin recep
tor) was indirectly mapped to human Xq28 by Jans et al.\textsuperscript{18} and Van Den Ouweland et al.,\textsuperscript{11} providing compelling support for the notion of a receptor mutation in NDI.

The human V\textsubscript{2} receptor has now been cloned by Birnbaumer et al.\textsuperscript{1} by a genomic expression approach. These investigators introduced human DNA into mouse L cells and screened for an adenylyl cyclase response to vasopressin exposure. DNA from cells that exhibited such a response, putatively due to the incorporation and expression of the human V\textsubscript{2} receptor gene, was again introduced into fresh L cells, to dilute out extraneous human DNA, and the DNA of those cells again acquiring vasopressin responsiveness, cloned into cosmid and phage. Recombinant clones were screened for the repetitive human DNA sequence Alu, to distinguish human from mouse DNA and the human clones tested for the ability to confer vasopressin responsiveness to L-cells, as did the original human DNA. Clones with this ability were used to screen a human renal cDNA library and a 1.7 kb cDNA was isolated. The deduced protein sequence of this gene predicts a transmembrane receptor protein with similarity to the superfamily of G-protein coupled receptors and to other ADH receptors (smooth muscle or vasopressin V\textsubscript{1} receptor and the oxytocin receptor).

Lolait et al.,\textsuperscript{2} independently cloned the rat V\textsubscript{2} receptor based upon the known sequence of the rat V\textsubscript{1} receptor using degenerate primers for the polymerase chain reaction. The use of degenerate primers, which include all possible DNA sequences predicted from a single amino acid sequence, is a useful approach to identify structurally similar or functionally related genes within or among species. A cDNA that identifies a 2.2 kb message on Northern analysis was found, and the predicted amino acid sequence predicts a G-like transmembrane protein. The similarity between the cDNA sequences identified by Birnbaumer et al. and Lolait et al. is very high, as would be expected between the human and rat versions of similar genes. Therefore, the mapping of Lolait et al. of the rat gene to human Xq27-pter firmly maps the human vasopressin V\textsubscript{2} receptor to the region known to contain the NDI locus and makes the gene an extremely good candidate gene for this disorder.

By sequencing normal and NDI vasopressin V\textsubscript{2} receptor genes, the candidacy of this gene for the NDI locus will be confirmed if mutation(s) are present. Of particular interest will be whether or not North American patients exhibit the same mutation, thus confirming the “Hopewell hypothesis.”

REFERENCES


Dr. Stephen T. Warren is Associate Investigator of the Howard Hughes Medical Institute, and Associate Professor of Biochemistry and of Pediatrics at Emory University School of Medicine in Atlanta. Prior to joining the Emory faculty in 1985 he was an NIH postdoctoral fellow in the Department of Genetics at the University of Illinois College of Medicine.

Dr. Warren received his Ph.D. in human genetics in 1981 from Michigan State University where he divided his time among the laboratory, conducting research on somatic cell genetics, and the clinic, seeing a wide variety of dysmorphologies and genetic diseases.

A diplomate of the American Board of Medical Genetics and a member of the Human Genome Organization involved in the mapping and sequencing of the human genome, he has held several positions within the American Society of Human Genetics. Currently he is associate editor of the American Journal of Human Genetics and serves on the editorial board of Human Molecular Genetics. Dr. Warren has published widely in human molecular genetics. Most recently he led an international team of scientists who discovered the gene responsible for the fragile X syndrome, the most common form of inherited mental retardation.