MOLECULAR BIOLOGY OF DIABETES INSIPIDUS

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ABSTRACT

The identification, characterization, and mutational analysis of three different genes, namely the prepro-arginine-vasopressin-neurophysin II gene (prepro-AVP-NPII), the arginine-vasopressin receptor 2 gene (AVPR2), and the vasopressin-sensitive water channel gene (aquaporin-2, AQP2), provide the basis for our understanding of three different hereditary forms of diabetes insipidus: autosomal dominant neurogenic diabetes insipidus, X-linked nephrogenic diabetes insipidus, and autosomal recessive nephrogenic diabetes insipidus, respectively. These advances provide diagnostic tools for physicians caring for these patients.

INTRODUCTION

Anyone passing large volumes of urine might be said to be suffering from diabetes insipidus. Years ago, the initial distinction made by physicians in evaluating patients with polyuria was whether their urine was sweet (diabetes
mellitus) or not (diabetes insipidus) (1). Diabetes insipidus is a disorder characterized by the excretion of abnormally large volumes (30 ml/kg of body weight per day for adult subjects) of dilute urine (<250 mmol/kg). Three basic defects can be involved: (a) deficient secretion of the antidiuretic hormone arginine vasopressin (AVP), which is the most common defect and is referred to as neurogenic (or central, or hypothalamic) diabetes insipidus; (b) renal insensitivity to the antidiuretic effect of AVP, which is known as nephrogenic diabetes insipidus; and (c) excessive water intake that can result in polyuria, which is referred to as primary polydipsia. The hereditary forms of diabetes insipidus account for less than 10% of the cases of diabetes insipidus seen in clinical practice. The purpose of this review is to examine recent developments in the understanding and molecular biology of the hereditary forms of diabetes insipidus.

GENES INVOLVED IN DIABETES INSIPIDUS

The regulation of the release of AVP from the posterior pituitary is primarily dependent, under normal circumstances, on tonicity information relayed by osmoreceptor cells in the anterior hypothalamus (for review see Ref. 2). AVP and its corresponding carrier, neurophysin II (NPII), are synthesized as a composite precursor by the magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus (for review see Ref. 3). The precursor is packaged into neurosecretory granules and transported axonally in the stalk of the posterior pituitary. En route to the neurohypophysis, the precursor is processed into the active hormone. Prepro-vasopressin has 164 amino acids and is encoded by the 2.5-kb prepro-AVP-NPII gene located in chromosome region 20p13 (4, 4a). Exon 1 of this gene encodes the signal peptide AVP and the NH2-terminal region of NPII. Exon 2 encodes the central region of NPII, and exon 3 encodes the COOH-terminal region of NPII and the glycopeptide. Pro-vasopressin is generated by the removal of the signal peptide from prepro-vasopressin and from the addition of a carbohydrate chain to the glycopeptide. Additional posttranslation processing occurs within neurosecretory vesicles during transport of the precursor protein to axon terminals in the posterior pituitary, yielding AVP, NPII, and glycopeptide. The AVP-NPII complex forms tetramers that can self-associate to form higher oligomers (5).

The first step in the action of AVP on water excretion is its binding to arginine-vasopressin type 2 receptors (V2 receptors) on the basolateral membrane of the collecting duct cells. The human V2 receptor gene, AVPR2, is located in chromosome region Xq28 and has three exons and two small introns (6, 7). The sequence of the cDNA predicts a polypeptide of 371 amino acids with a structure typical of guanine-nucleotide (G) protein-coupled receptors with seven transmembrane, four extracellular, and four cytoplasmic domains
The activation of the V2 receptor on renal collecting tubules stimulates adenylyl cyclase via the stimulatory G protein (Gs) and promotes the cyclic adenosine monophosphate (cAMP)–mediated incorporation of water pores into the luminal surface of these cells. This process is the molecular basis of the vasopressin-induced increase in the osmotic water permeability of the apical membrane of the collecting tubule. The gene that codes for the water channel of the apical membrane of the kidney-collecting tubule has been designated aquaporin-2 (AQP2) and was cloned by homology to the rat aquaporin of collecting duct (11, 12, 13).
human AQP2 gene is located in chromosome region 12q13 and has four exons and three introns (12, 13, 14). It is predicted to code for a polypeptide of 271 amino acids that is organized into two repeats oriented at 180° to each other and that has six membrane-spanning domains, with both terminal ends located intracellularly, and conserved Asn-Pro-Ala boxes (Figure 2). These features are characteristic of the major intrinsic protein family (13). There is 48% amino acid sequence identity between AQP2 and the human channel–forming integral protein of 28 kDa (CHIP28 or aquaporin-1), a water channel in erythrocytes and in the kidney proximal and descending tubules (15).

Figure 2  Schematic representation of the aquaporin-2 protein and identification of four AQP2 mutations (12, 12a).

aThe names were assigned following the suggested nomenclature for mutations (9). The nucleotides and amino acids are numbered according to the prepro-AVP-NPII gene sequence published by Sausville et al (4) and to GenBank accession number M11166. The codons corresponding to the moieties are 1–19, signal peptide; 20–28, AVP; 29–31, cleavage site; 32–124, NPII; and 126–164, glycopeptide. The amino acids of AQP2 are numbered according to Refs. 12 and 12a. The nucleotides, which are numbered according to GenBank accession number Z29491 (excluding the partial introns), correspond to the nucleotide number plus 70 in Figure 2 of Ref. 12a.
<table>
<thead>
<tr>
<th>Name</th>
<th>Type of mutation</th>
<th>Nucleotide change</th>
<th>Predicted amino acid change</th>
<th>Restriction-enzyme analysis</th>
<th>Comments and putative functional consequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A19T</td>
<td>Missense</td>
<td>G → A at nucleotide 279</td>
<td>Ala → Thr at codon 19</td>
<td><em>BstUI</em> site abolished</td>
<td>CG → CA; alteration of the cleavage of the leader peptide</td>
<td>20–22</td>
</tr>
<tr>
<td>G48V</td>
<td>Missense</td>
<td>G → T at nucleotide 1740</td>
<td>Gly → Val at codon 48</td>
<td><em>BglII</em> site abolished</td>
<td>Disruption of a β turn in AVP-NPII precursor</td>
<td>19</td>
</tr>
<tr>
<td>ΔE77</td>
<td>Inframe deletion</td>
<td>Deletion of 3 nucleotides in region 1824–1831</td>
<td>Deletion of Glu at codon 77</td>
<td><em>MnlI</em> site abolished</td>
<td>3 sets of staggered 3 bp tandem repeats; unable to form a salt bridge between AVP and NPII</td>
<td>23</td>
</tr>
<tr>
<td>G88S</td>
<td>Missense</td>
<td>G → A at nucleotide 1859</td>
<td>Gly → Ser at codon 88</td>
<td><em>MspI</em> site abolished</td>
<td>Failure of dimerization of NPII, alteration of axonal transport or posttranslational processing</td>
<td>18</td>
</tr>
<tr>
<td>G64R</td>
<td>Missense</td>
<td>G → A at nucleotide 260</td>
<td>Gly → Arg at codon 64</td>
<td></td>
<td>CG → CA</td>
<td>12a</td>
</tr>
<tr>
<td>439delC</td>
<td>Frameshift</td>
<td>Deletion of C at nucleotide 439</td>
<td>Frameshift 3’ to codon 123; codon 131 → stop</td>
<td></td>
<td></td>
<td>12a</td>
</tr>
<tr>
<td>R187C</td>
<td>Missense</td>
<td>C → T at nucleotide 629</td>
<td>Arg → Cys at codon 187</td>
<td></td>
<td>CG → TG</td>
<td>12, 12a</td>
</tr>
<tr>
<td>S216P</td>
<td>Missense</td>
<td>T → C at nucleotide 716</td>
<td>Ser → Pro at codon 216</td>
<td><em>ApaI</em> site created</td>
<td>Distortion of the α-helical structure</td>
<td></td>
</tr>
</tbody>
</table>
AUTOSOMAL DOMINANT NEUROGENIC DIABETES INSIPIDUS AND MUTATIONS IN THE PREPRO-AVP-NPII GENE

The classical animal model for studying diabetes insipidus has been the Brattleboro rat with autosomal recessive diabetes insipidus, \( \text{di/di} \) (16). This form of hereditary diabetes insipidus is not found in humans. \( \text{di/di} \) rats are homozygous for a 1-bp deletion in the second exon that results in a frameshift mutation in the coding sequence of the carrier NPII. The mutant allele encodes a normal AVP but an abnormal NPII moiety that impairs transport and processing of the AVP-NPII precursor and results in retention of the abnormal polypeptide in the endoplasmic reticulum of the magnocellular cells (17).

Four prepro-AVP-NPII mutations segregating with autosomal dominant neurogenic diabetes insipidus have been described (18–23; Table 1). The mechanism(s) by which a mutant allele causes neurogenic diabetes insipidus could be a gain of function of the mutant protein, a loss of function due to insufficient normal product, or an interaction of the mutant and normal product by degradation, functional inactivation of an oligomer, or interference with processing. If defective protein slowly accumulates and results in the death of magnocellular neurons, this could explain the absence of symptoms in the first years of life of a heterozygote, when expression of the normal allele is sufficient to mount an appropriate antidiuretic response (20, 25). The absence of symptoms in infancy is in sharp contrast to X-linked and probably also to autosomal recessive nephrogenic diabetes insipidus, in which the polyuric-polydipsic symptoms are present during the first week of life.

X-LINKED NEPHROGENIC DIABETES INSIPIDUS AND MUTATIONS IN THE AVPR2 GENE

X-linked nephrogenic diabetes insipidus [MIM 304800 (26)] is generally a rare disease in which the affected male patients do not concentrate their urine after administration of AVP (27). Because this form is a rare, recessive X-linked disease, females are unlikely to be affected, but heterozygous females exhibit variable degrees of polyuria and polydipsia because of X inactivation. In Quebec, the incidence of this disease among males was estimated to be approximately 4 in 1,000,000 (28). A founder effect for a particular \( \text{AVPR2} \) mutation in Ulster Scot immigrants resulted in an elevated prevalence of X-linked NDI in their descendants and was estimated to afflict approximately 24 in 1000 males in certain communities in Nova Scotia. The W71X mutation was identified as the cause of NDI in the extended kindred studied by Bode & Crawford (29; the so-called Hopewell kindred) and in families in the Canadian Maritime provinces (10a, 31). Among X-linked NDI patients in North Amer-
ica, the W71X mutation is more common than any other AVPR2 mutation. The W71X mutations of these patients are likely identical by descent, although a common ancestor has not been identified in all cases.

The natural history of untreated disease includes hypernatremia, hyperthermia, mental retardation, and repeated episodes of dehydration in early infancy. Mental retardation is a consequence of repeated episodes of dehydration and was present in 73 of 82 patients (89%) in the Crawford & Bode study (32). Early recognition and treatment of X-linked NDI with an abundant intake of water allow a normal life span with normal physical and mental development (33; DG Bichet, personal observations).

Sixty-three (8a, 10a–10i) putative disease-causing mutations in AVPR2 have now been reported in 90 presumably unrelated families with X-linked NDI from diverse ethnic groups (see Figure 1). In X-linked NDI, loss of mutant alleles from the population occurs because of the higher mortality of affected males compared with normal males, whereas gain of mutant alleles occurs by mutation. If affected males with a rare X-linked recessive disease do not reproduce and if mutation rates are equal in mothers and fathers, then, at genetic equilibrium, one third of new cases of affected males will be due to new mutations (34). The gametic origins of new mutations could be identified or inferred in 18 families and included 5 maternal, 9 grandpaternal, and 2 grandmaternal gametes, 1 great-grandmaternal gamete, and 1 gamete that was either of grandpaternal or grandmaternal origin (8a, 10c, 10d, 35). Figure 3 illustrates the identification of a new mutation.
Eleven different AVPR2 mutations were observed in more than one independent NDI family, suggesting that there are hot spots for mutations. Additional evidence for recurrent mutation is the observation that the mutation occurs on different haplotypes defined by markers in or very close to the AVPR2 gene (8a, 10c). Seven of the 11 mutations (V88M, R113W, R137H, S167L, R181C, R202C, and R337X) were single nucleotide substitutions at a CpG dinucleotide. Methylated CpG dinucleotides are recognized hot spots for mutation (36). Thirteen of 18 small deletion or insertion mutations (1 to 35 bp)
involved direct or complementary repeats (2 to 9 bp) or strings of 4 to 6 guanines (see Figure 2 or Ref. 8a and Figure 4). This finding suggests that these deletions, like many others that have been described in other genes, resulted from DNA strand slippage and mispairing during replication (36–39).

The cause of loss of function of mutant V2 receptors has been studied in vitro expression systems. Truncated polypeptides, which are expected to be nonfunctional, or absent protein is expected in the case of nonsense mutations (W71X, Q119X, Y124X, Q225X, E231X, E242X, W284X, W293X, L312X, and R337X). A missense mutation could result in misfolding of the protein and trapping in the endoplasmic reticulum or in alteration of the binding pocket or the contact surface that activates the stimulatory G protein. The R137H mutant protein expressed in cultured kidney cells from African green monkey (COS cells) from transfected mutant cDNA exhibited a normal binding affinity for AVP but failed to stimulate the Gs/adenyl cyclase system (40). The R113W mutant cDNA was also transfected into COS cells, and the mutant protein exhibited a combination of functional defects: lowered affinity for vasopressin, diminished ability to stimulate adenyl cyclase, and diminished ability to reach the cell surface (41). No adenyl cyclase activity was detected in COS cells transfected with cDNA of a nonsense mutation (Q119X), a frameshift mutation (763delA or 855delG), or missense mutations (10i). In addition, functional analysis of AVPR2 mutations indicated that the 810del12 mutation was not the cause of NDI in a patient who had this mutation as well as the R181C mutation (10i, 35). The R181C mutant receptor had less than half of the normal adenylyl cyclase activity, and although it was expressed at the same level as the normal V2 receptor, the EC50 for adenylyl cyclase stimulation was increased, presumably owing to its altered structure.

AUTOSOMAL RECESSIVE NEPHROGENIC DIABETES INSIPIDUS AND MUTATIONS IN THE AQP2 GENE

On the basis of (a) phenotypic characteristics of both males and females affected with NDI and (b) dDAVP infusion studies, a non-X-linked form of NDI with a postreceptor defect was suggested (42–45). In contrast to male patients affected with X-linked NDI (46, 47), two male and two female NDI patients experienced a normal rise, e.g. two- to threefold, in the plasma concentrations of factor VIII and von Willebrand factor after dDAVP infusion, but urinary osmolality remained low (42). X-linked NDI was excluded for two sisters with vasopressin-resistant hypotonicity because they inherited different alleles of an Xq28 marker from their mother; autosomal recessive inheritance was suggested on the basis of gender, parental consanguinity, and normal urine concentration in the parents (44). Two male patients were described who had normal stimulation of plasma cAMP after dDAVP administration but in
whom no AVPR2 mutation was identified (45). A patient who presented shortly after birth with typical features of NDI but who exhibited normal coagulation and normal fibrinolytic and vasodilatory responses to dDAVP was recently shown to be a compound heterozygote for two missense mutations (R187C and S217P) in the AQP2 gene (12, 43; Figure 2 and Table 1). Three NDI patients from consanguineous matings were found to be homozygous for missense mutations (R187C or G64R) or a frameshift mutation (369delC) in the AQP2 gene. Functional expression studies showed that Xenopus oocytes injected with mutant cRNA had abnormal coefficients of water permeability, while Xenopus oocytes injected with both mutant and normal cRNAs had coefficients of water permeability similar to those of normal constructs alone (12, 12a). These findings provide conclusive evidence that NDI can be caused by homozygosity for mutations in the AQP2 gene. The increased frequency of consanguinity between normal parents of affected children of both genders with AQP2 mutations is consistent with autosomal recessive inheritance of a rare genetic disease.

CARRIER DETECTION AND POSTNATAL DIAGNOSIS

The identification of mutations in three genes that cause diabetes insipidus allows the early diagnosis and management of at-risk members of families with identified mutations. We encourage physicians who follow families with X-linked NDI to recommend mutation analysis before the birth of a male infant because early diagnosis and treatment of male infants can avert the physical and mental retardation associated with episodes of dehydration. Diagnosis of X-linked NDI within 48 h of birth was accomplished by mutation testing of a sample of cord blood (8a). Early diagnosis of autosomal recessive NDI is also essential for early treatment of affected infants in order to avoid repeated episodes of dehydration. Mutation detection in families with inherited neurogenic diabetes insipidus provides a powerful clinical tool for early diagnosis and management of subsequent cases, especially in early childhood, when diagnosis is difficult and the clinical risks are the greatest (25).

SUMMARY

It is hoped that an understanding of the pathophysiology of defects in the antidiuretic hormone, the V2 receptor, and the water channel due to mutations in the prepro-AVP-NPII, AVPR2, and AQP2 genes, respectively, will provide insight into the more numerous cases of sporadic diabetes insipidus. In turn, the study of diabetes insipidus caused by mutations in these and additional genes will increase our knowledge of normal vasopressin-regulated water transport across the kidney epithelium.
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