Inheritance of mutations in the \( V_2 \) receptor gene in thirteen families with nephrogenic diabetes insipidus

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Inheritance of mutations in the \( V_2 \) receptor gene in thirteen families with nephrogenic diabetes insipidus. Congenital nephrogenic diabetes insipidus (NDI) is an X-linked recessive disease characterized by insensitivity of the distal nephron to the antidiuretic effect of arginine vasopressin. The hypothesis that the defect underlying NDI might be a dysfunctional renal vasopressin \( V_2 \) receptor has recently been proven by the identification of mutations in the \( V_2 \) receptor gene in NDI patients. We examined thirteen unrelated Dutch NDI families and identified thirteen distinct and unique mutations. These included nine missense mutations, two nonsense mutations and two small deletions and were found in the extracellular domains II, III and IV, the intracellular domains I and IV and in the transmembrane loops I, II, IV and V of the vasopressin type 2 receptor. In the families with multiple NDI patients the mutated gene cosegregated with the disease. Our data suggest a higher mutation frequency in male than in female gametes. No discrepancies between carrier detection by means of DNA analysis with closely linked polymorphic markers and the definite diagnosis based on sequencing data were found.

Congenital nephrogenic diabetes insipidus (NDI) is mostly an X-linked recessive disease, characterized by renal resistance to the antidiuretic effect of arginine vasopressin. The disorder becomes manifest early in infancy with polyuria, periods of life-threatening dehydration, fever, anorexia, vomiting and failure to thrive. Without treatment, mental retardation may develop probably due to brain dehydration. Until recently, the molecular basis underlying NDI was unknown. The neurohypophyseal hormone arginine vasopressin (AVP) exerts its physiological effects through stimulation of at least two distinct G-protein-coupled receptors, vasopressin \( V_1 \) and \( V_2 \) receptors [1]. The vascular \( V_1 \) receptor, which is connected to a phosphoinositide specific phospholipase and \( Ca^{2+} \) mobilization, mediates vasoconstrictor and glycoegenolytic responses; the renal tubular \( V_2 \) receptor, which is connected to the membrane-bound adenylate cyclase and stimulation of cyclic AMP, mediates the antidiuretic response to vasopressin.

In addition to mediating antidiuresis, an apparent \( V_2 \) mediated action of AVP and its \( V_2 \)-specific agonist, 1-desamino-8-D-arginine vasopressin (DDAVP), causes release of coagulation and fibrinolytic factors [2] and vasodilation [3]. These extra-renal responses are also absent in NDI patients [4–6], suggesting that the defect in this disorder might be a dysfunctional or nonfunctional vasopressin \( V_2 \) receptor.

In the last five years the understanding of the genetics and pathogenesis of X-linked NDI has expanded considerably. The beginning of the era of studying NDI was marked by the localization of the disease gene to the distal region of the X chromosome long arm, to Xq28 [7, 8]. The next step was the discovery of functional vasopressin \( V_2 \) receptor activity in somatic cell hybrids that carried at least the terminal region of the human X chromosome (Xqter) [9, 10]. This finding supported the assumption that the NDI gene and the vasopressin \( V_2 \) receptor gene might be identical. Very recently, the cloning of the \( V_2 \) receptor gene from human [11] and rat [12] was reported. The \( V_2 \) receptor gene encodes a G-protein coupled receptor protein containing seven putative transmembrane helices with considerable sequence homology to the \( V_1 \) vasopressin and oxytocin receptors.

Definite proof for a vasopressin \( V_2 \) receptor defect being the cause of NDI was obtained with the identification of mutations in the \( V_2 \) receptor gene in NDI patients [13–18].

In this article we report mutations in the \( V_2 \) receptor gene in 13 Dutch NDI families. The implications for genetic counseling are discussed.

Methods

Study subjects

Thirteen NDI families from the Netherlands were included in the study. All patients were males, eight of them being sporadic cases, whereas in five families transmission was compatible with X-linkage. Diagnosis of NDI was based on clinical symptoms and lack of increase of urinary osmolality after administration of the synthetic vasopressin analogue DDAVP. Maximal urine osmolalities that could be achieved after DDAVP infusion were 51 to 198 mOsmol/kg (normal > 805 mOsmol/kg).

DNA analysis

Procedures for the isolation of genomic DNA from patient blood cells as well as Southern blotting analysis have been described elsewhere [7, 8]. The additional markers tested were an MspI polymorphism at the locus DXS304 [19], a BstYI polymorphism at the locus DXS455 [20], a TaqI polymorphism at the locus DXS305 [21], an MspI polymorphism at locus

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Table 1. Mutations in the V2 receptor gene in 13 unrelated families with nephrogenic diabetes insipidus

<table>
<thead>
<tr>
<th>Family</th>
<th>DNA mutation</th>
<th>Mutation at the protein level</th>
<th>Domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>029/ND</td>
<td>C377T</td>
<td>Arg113Trp</td>
<td>EI1</td>
</tr>
<tr>
<td>018/ND</td>
<td>C611A</td>
<td>Thr204Asn</td>
<td>EI1</td>
</tr>
<tr>
<td>070/ND</td>
<td>T617A</td>
<td>Val206Asp</td>
<td>EI1</td>
</tr>
<tr>
<td>362/ND</td>
<td>C541T</td>
<td>Arg181Cys</td>
<td>EI1</td>
</tr>
<tr>
<td>763/ND</td>
<td>906delC</td>
<td>frameshift</td>
<td>EIV</td>
</tr>
<tr>
<td>024/ND</td>
<td>457delC</td>
<td>frameshift</td>
<td>CIV</td>
</tr>
<tr>
<td>023/ND</td>
<td>C1099T</td>
<td>Arg337STOP</td>
<td>CIV</td>
</tr>
<tr>
<td>027/ND</td>
<td>C130T</td>
<td>Leu44Phe</td>
<td>TM1</td>
</tr>
<tr>
<td>982/ND</td>
<td>T185C</td>
<td>Leu62Pro</td>
<td>TM1</td>
</tr>
<tr>
<td>025/ND</td>
<td>G253A</td>
<td>Asp85Asn</td>
<td>TM2</td>
</tr>
<tr>
<td>456/ND</td>
<td>G262A</td>
<td>Val88Met</td>
<td>TM2</td>
</tr>
<tr>
<td>046/ND</td>
<td>C500T</td>
<td>Ser167Leu</td>
<td>TM4</td>
</tr>
<tr>
<td>013/ND</td>
<td>C673T</td>
<td>Gln225STOP</td>
<td>TM5</td>
</tr>
</tbody>
</table>

- **a** Amino acid numbering according to Birnbaumer et al [11]
- **b** Abbreviations are: E, extracellular domain; C, intracellular domain; TM, transmembrane region of the proposed topology of the V2 receptor protein [11, 12]

Fig. 1. Map of the X chromosome showing the position of the Xq28 markers used in this study in relation to the position of the NDI/V2 receptor gene. The order of loci is derived from the latest X chromosome workshop report [24]. Abbreviation is AVPR2, vasopressin V2 receptor gene.

DXS707, an EcoRI polymorphism at the DXS605 locus [22], and a CA repeat at the F8 locus [23]. The allele sizes of most of these polymorphisms are listed in the last Human Gene Mapping report [24] and the localization of the markers with respect to the NDI locus is depicted in Figure 1. Direct automated DNA sequence analysis on both strands of genomic patient DNA was performed as described previously [17].

Results

The mutations in V2 receptor gene found in the probands of each family are shown in Table 1. Thirteen distinct mutations were identified: two small deletions, two nonsense mutations and nine missense mutations. These mutations are not clustered in one domain of the V2 receptor protein but are found throughout almost the whole receptor, in the extracellular domains II, III, and IV, the intracellular domains II and IV, and the transmembrane loops I, II, IV, and V. After the mutation in the affected probands in each family had been characterized, other family members were screened either by restriction enzyme analysis (for example, family 024/ND, Fig. 2) or, when this was not possible by direct sequencing of genomic DNA. In each of the five families with multiple NDI patients, the mutation identified in the proband was found in all other patients but not in the unaffected males of that particular family.

In seven families NDI was due to a de novo mutation. By combining haplotypes of DNA markers flanking the vasopressin V2 receptor gene with mutation data in each of these seven families, the origin of the mutation was deduced in six of them. In four families the mutation occurred most likely during male

Fig. 2. Segregation of a point mutation in the V2 receptor gene in family 024/ND followed by restriction enzyme digestion. The mutation found in the proband resulted in the loss of a BsrI site. The region around the mutation was amplified with the primers 5'TGGCTCTGTCTCAAGTGCCTG3' (position 263-282 [11]) and 5'GAGGTGACATAGGTGGACG3' (position 603-623 [11]). PCR was performed as described previously [17]. The PCR product was digested with BsrI and the fragments were resolved on a 3% agarose gel and stained with ethidium bromide. For the mutant allele the PCR product of 360 bp was not cleaved by BsrI, while the wild type allele gave two fragments. The sizes of the PCR fragments as deduced by comparison with DNA fragment length standards (not shown) were in agreement with the sizes predicted on the basis of the published V2 receptor cDNA sequence. The mother of the patient shows three bands, indicative for heterozygosity. In the lanes of the PCR fragments of the wild type allele a very faint band at the position of the uncleaved PCR product is still visible, which is of no significance. Solid symbol represents the affected individual.
Fig. 3. Pedigrees of seven families with nephrogenic diabetes insipidus (NDI), in which NDI appeared to be due to a new mutation. Pedigrees 023/ND, 024/ND, 027/ND, and 046/ND have been published before in less detail [7, 8]. Indicated are the alleles for the DNA markers DXS304 (pU6.2: B,b), DXS455 (p346.72:M,m), DXS305 (pSt35.69:F,f), DXS52 (pSt14: 1,2,3,4,5,6), DXS15 (pDX13: D,d), DXS707 (p2-55: G,g), CB (phs7:H,h), DXS605 (p2-19:A,a), and F8 disease is associated with the 1-d-H-E haplotype in pedigree 024/ND, with the b-6-H-E haplotype in pedigree 023/ND, with the m-6-H-2 haplotype in pedigree 018/ND, with the D-E haplotype in pedigree 027/ND, with the 1-A2 haplotype in pedigree 456/ND and with the 2-e haplotype in pedigree 046/ND. The segregation of the mutation in the vasopressin V2 receptor gene (AVPR2) in each family is depicted by - for the normal allele and + for the mutant allele. Solid symbols represent affected individuals and obligate carriers are indicated by a dot in the symbol; n.t is not tested.
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C Pedigree 018/ND

Fig. 3. Continued
meiosis: during spermatogenesis in the maternal grandfather in three families (Fig. 3 a, b, c) and in the maternal great-grandfather in one family (Fig. 3d). In two families the mutation arose during female meiosis: during oogenesis in the mother in one family (Fig. 3e) and in the maternal grandmother in the other family (Fig. 3f). In family 046/ND (Fig. 3g) the origin of the mutation could not be determined, either during spermatogenesis in the maternal grandfather or during oogenesis in the maternal grandmother.

Discussion

The assumption that the NDI gene might be identical to the vasopressin V₂ receptor gene has recently been confirmed by the identification of several mutations in the V₂ receptor gene in...
s of NDI is necessary to define the exact mutation frequency for NDI in male and female gametes. Knowledge of the sex ratios of mutation frequencies has implications for genetic counseling in families with isolated cases. If the mutation frequency is indeed higher in males than in females, a high proportion of the mothers of isolated patients will be carriers of the gene.

In summary, we identified thirteen distinct and unique mutations in thirteen unrelated NDI families. Our data suggest the possibility of a higher mutation frequency in males than in females, and underscore the reliability of linkage analysis for heterozygote detection in NDI.

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