Molecular and cellular defects in nephrogenic diabetes insipidus

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Nephrogenic diabetes insipidus is a rare genetic disorder characterized by insensitivity of the distal nephron to the antidiuretic effect of arginine vasopressin. Two different molecular defects underlying this disease have so far been identified. Mutations in the gene encoding the vasopressin type-2 receptor cause the X-chromosomal form of the disease, whereas mutations in the gene encoding the vasopressin-dependent water channel aquaporin-2 are responsible for the autosomal recessive, and (in some cases) an autosomal dominant type of the disease. Functional analysis of naturally occurring mutations in the vasopressin type-2 receptor and aquaporin-2 have increased the insight into the structure and function of both proteins and have led to substantial progress in understanding the cellular mechanisms underlying the concentrating ability of the kidney. Some female carriers of a vasopressin type-2 receptor mutation may show complete manifestation of nephrogenic diabetes insipidus, probably as a result of skewed X-inactivation. The recent findings in nephrogenic diabetes insipidus research have considerable impact for diagnosis of and genetic counselling for this disease.

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Abbreviations

AQP2	aquaporin 2
AVP	arginine vasopressin
DDAVP	1-deamino-8-D-arginine vasopressin
NDI	nephrogenic diabetes insipidus
V_2	vasopressin type 2

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Introduction

Nephrogenic diabetes insipidus (NDI) is an inherited kidney disorder in which the renal concentrating mechanism fails because of insensitivity of the collecting tubule to circulating arginine vasopressin (AVP). Consequently, large volumes of hypotonic urine are excreted, which may lead to severe dehydration. Patients present in their first year of life with aspecific symptoms such as anorexia, vomiting, fever, growth retardation and developmental delay. After infanthood the clinical picture is dominated by the less alarming symptoms polyuria and polydipsia. Mental retardation has long been considered an important complication of untreated NDI and assumed to be a sequel of severe brain dehydration. However, in a recent psychological study in 17 patients with NDI we found that the prevalence of mental retardation is considerably lower than is suggested in the literature [1]. This is probably attributable to the earlier recognition and better treatment of the disorder.

NDI is genetically heterogeneous. In most cases the disease is transmitted as an X-linked recessive trait, but in some families an autosomal mode of inheritance has been found. Families with either form have been reported. In the past 4 years two different gene defects causing the NDI phenotype have been identified. Functional analysis of these molecular defects has contributed considerably to a better understanding of the cellular mechanisms underlying AVP-induced antidiuresis.

In this review we briefly discuss the present knowledge of the AVP-signalling cascade in the renal medulla and give an updated overview of the molecular causes of NDI.

Pathway of the action of vasopressin in the renal collecting duct

Normally, AVP is secreted from the neurohypophysis in response to an increase in serum osmolality or to hypovolaemia. The hormone binds to vasopressin type-2 (V_2) receptors at the outer surface of basolateral membranes of the principal and inner medullary collecting duct cells. The human V_2 receptor cDNA was cloned in 1992 and encodes a 371-amino-acid G-protein-coupled receptor containing seven putative transmembrane helices with considerable sequence homology to the vasopressin type-1 and oxytocin receptors [2]. V_2 receptor occupancy results, via the intermediacy of a stimulatory G-protein, in activation of adenylate cyclase and an increase in intracellular cAMP levels. In turn, by an unknown mechanism in which protein kinase A and the cytoskeleton are involved, cAMP initiates the insertion of intracellular

vesicles containing water channels into the apical membrane. This causes a dramatic increase in water permeability and allows water to flow from the tubular lumen to the hypertonic medullary interstitium. When stimulation by AVP is terminated, the water channels are removed from the apical membrane by endocytosis of the vesicles. This controlled movement of vesicles between the apical membrane and the cytoplasm is known as the 'membrane shuttle' mechanism [3]. In 1993 the cDNA for an apical membrane water channel of the rat renal collecting duct was cloned [4]. The gene encodes a 271-amino-acid protein which belongs to a family of membrane integral proteins, now renamed aquaporins, which function as selective water transporters throughout the plant and animal kingdoms (for review [5,6,7••]). The water channel of the renal collecting duct, designated aquaporin-2 (AQP2), is exclusively localized in the apical membrane and a subapical compartment of collecting duct cells, and is upregulated by dehydration or AVP [8,9], suggesting that it is the AVP-regulated water channel. Support for the membrane shuttle mechanism was provided by immunolocalization and expression studies in isolated collecting ducts [10,11], which showed translocation of AQP2 from the subapical vesicles to the apical membrane and induction of water permeability after addition of AVP. In 1994 the human equivalent of the rat AQP2 gene was cloned independently by two different groups and assigned to chromosome 12 [12–14].

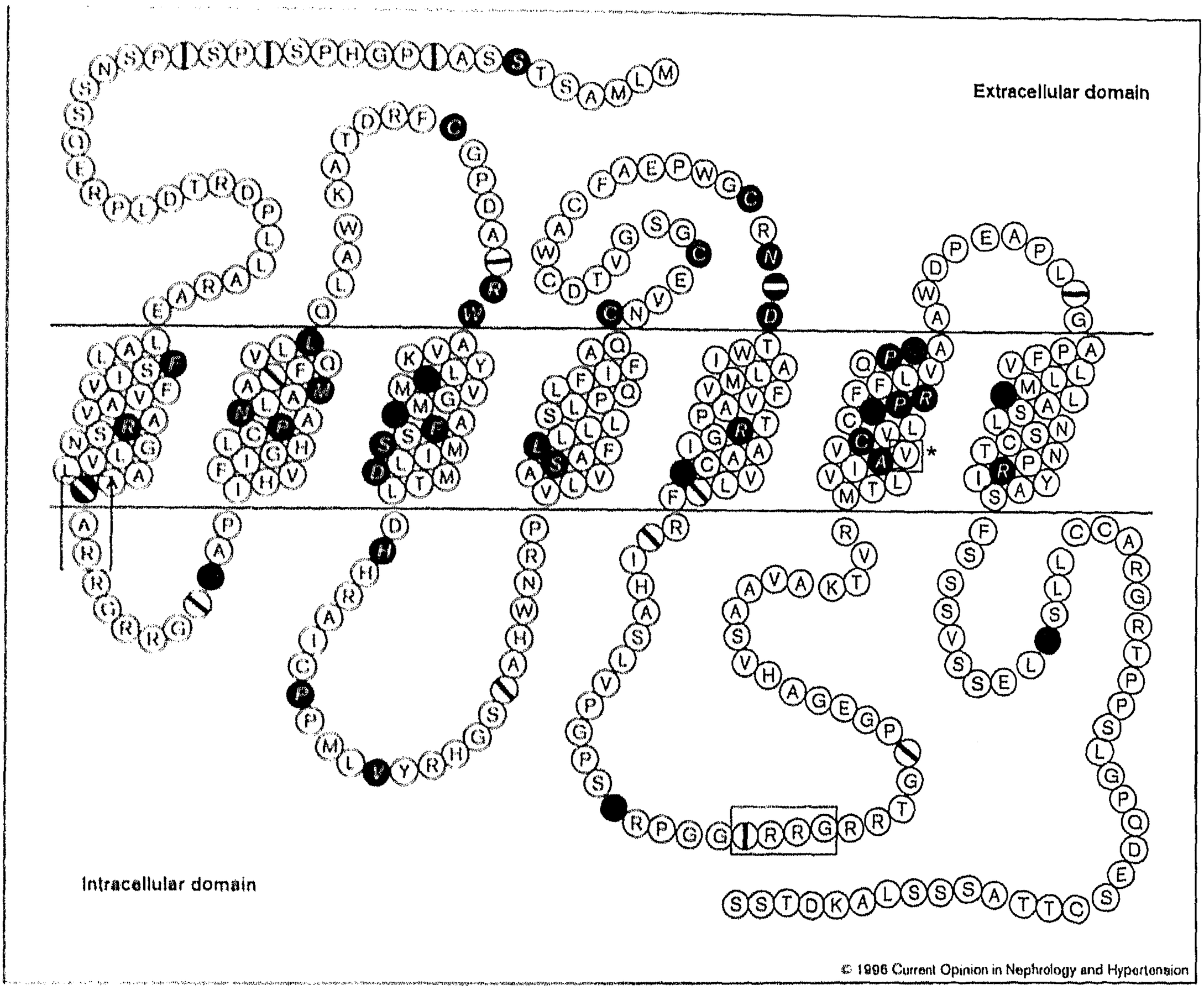
X-linked nephrogenic diabetes insipidus

The V_2 receptor has long been considered a prime candidate for the defective step in AVP-mediated response in X-linked NDI. The reason for this belief was that the V_2-specific agonist 1-desamino-8-D-arginine vasopressin (DDAVP), which normally elicits vasodilatory, coagulatory and fibrinolytic responses, does not exert these effects in males with X-linked NDI, suggesting a general V_2 receptor defect [15]. Independent support for the V_2 receptor being involved in X-linked NDI was provided by the finding that a gene conferring V_2-like binding activity colocalized with the NDI locus in the subterminal region of the long arm of the X chromosome (Xq28) [16]. The role of the V_2 receptor in the pathogenesis of NDI was finally proved by the demonstration of mutant V_2 receptor genes in affected individuals [17–19]. More than 60 distinct putative disease-causing mutations throughout the V_2 receptor gene have so far been reported in families with NDI [20,21,22•] (for review [23,24]) (Fig. 1). The impact of several mutations on the function of the V_2 receptor, especially important for the mis-sense mutations, has been studied in in-vitro expression systems. A total loss of binding of AVP was observed for the R202C, Y205C and V206D mutations in the third extracellular domain of the receptor [26,27], the Y128S and P286R mutations in the third and sixth transmembrane region, respectively [28], and several frameshift and non-sense mutations [28,29]. Conversely, the receptor with the R137H mutation, located at the junction of the third transmembrane region and the second intracellular loop, exhibited a normal binding activity for AVP but failed to stimulate the G_s adenylate cyclase system [30]. Using an exofacial epitope-tagging technique which facilitates surface localization of receptors, Tsukaguchi *et al.* [31••] reported evidence for a further heterogeneity of the cellular effects of NDI mutations. They demonstrated that the reduced ligand-binding activity of the R143P and V278del mutations is a result of blocked transport of the receptor to the cell surface, whereas the same functional defect of the non-sense 804insG mutant is caused by ineffective biosynthesis or accelerated degradation, or both, of the receptor protein [31••]. Interestingly, the R113W mutation in the first extracellular loop has been shown to cause a combination of defects: lowered affinity for AVP, reduced ability to stimulate adenylate cyclase and hindrance of transport of the receptor to the cell membrane [32]. A reduced ligand binding capacity has also been reported for the R181C mutation and the T204N mutation in the third extracellular domain [27,28]. An A61V mis-sense mutation in the first transmembrane region and an in-frame deletion of four amino acids in the third intracellular loop were shown not to cause NDI and probably represent rare polymorphisms [28]. Using site-directed mutagenesis, Sadeghi *et al.* [33] demonstrated that certain post-translational modifications of the V_2 receptor, namely glycosylation at asparagine 22 and palmitoylation at cysteines 341 and 342 are not critical for its function [33].

Autosomal recessive nephrogenic diabetes insipidus

In approximately 10% of families NDI shows a non-X-linked pattern of inheritance. In one patient from such a family a normal extrarenal response to DDAVP has been observed [34], indicating that the unresponsiveness to AVP in this patient was restricted to the kidney. Linkage between the NDI gene and polymorphic markers from the Xq28 region was excluded in the patient's family, and sequencing of the V_2 receptor gene did not identify a potentially harmful mutation. Sequencing of the AQP2 gene in this patient revealed that he was a compound heterozygote for two point mutations (R187C and S217P) in the AQP2 gene (Fig. 2) [12]. Subsequently, homozygous mutations in the AQP2 gene were found in three additional patients with NDI, all born from consanguineous parents [36]. Functional expression studies revealed that *Xenopus laevis* oocytes injected with mutant AQP2 cRNA failed to increase water permeability, because of impaired transport to the plasma membrane and concomitant degradation of the mutant AQP2 [12,36,37••,38]. Co-injection of mutant and wild-type AQP2 cRNA resulted in the same water permeability as injection of wild-type cRNA only, a finding in accord with the autosomal inheritance of NDI in the four families studied. Two

Figure 1

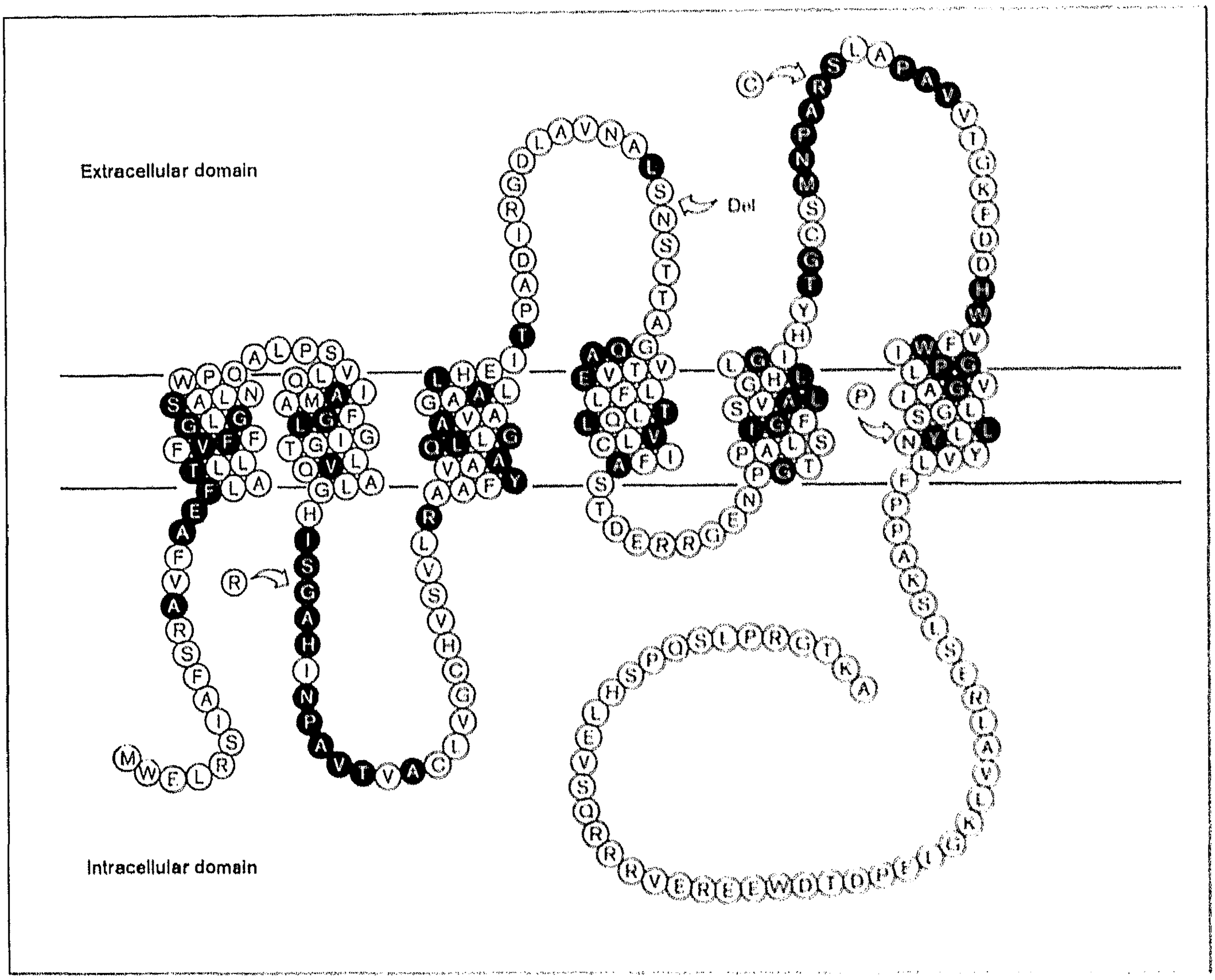


Model of the vasopressin V_2 receptor and location of the various mutations identified in X-linked nephrogenic diabetes insipidus. The predicted domain structure with seven transmembrane-spanning regions, four extracellular domains and four intracellular domains was taken from [25]. Solid symbols, non-sense mutations, solid symbols with mutated amino acids in italics, mis-sense mutations; bars, frameshift mutations; open squares, in-frame deletions *Deletion of valine at 278 or 279.

families with autosomal dominant transmission of NDI were recently reported [39], in which patients were heterozygous for a mutation in the AQP2 gene. The identified mutations are located in the carboxyl-terminal region of AQP2, a region considered to be important for targeting of proteins. Although the functional consequences of these mutations have not yet been examined, it is likely that they have a dominant negative effect, interfering with the transport or functioning, or both, of the wild-type AQP2. Patients with an AQP2 defect cannot be distinguished, on the basis of clinical symptomatology, from patients with a V_2 receptor defect. Prompted by the positive extrarenal reaction to DDAVP in the first patient with an AQP2 defect, van Lieburg *et al.* [40] tested fibrinolytic responses in three further patients with distinct AQP2 mutations. Based on the presence of normal reactions in all of the patients tested, they concluded that a DDAVP test allows these patients to be distinguished from those with a V_2 receptor defect.

AQP2 is detectable in the urine of normal individuals and, after treatment with DDAVP, in the urine of patients with central diabetes insipidus but not in that of patients with NDI [41•]. However, the significance of measuring urinary AQP2 for diagnostic purposes is not clear, especially because it cannot discriminate between X-linked and non-X-linked NDI [42].

Figure 2



Schematic representation of the aquaporin-2 protein. The positions of the single nucleotide deletion and amino acid substitutions found in the patients with autosomal recessive nephrogenic diabetes insipidus are indicated by arrows. Filled symbols represent amino acids that are conserved within the family of membrane integral proteins [35]. Reprinted by permission of the publisher from Molecular characterization of nephrogenic diabetes insipidus by N Knoers, *Trends Endocrinal Metab* 5:422–428. Copyright 1994 by Elsevier Science Inc [23].

Vasopressin insensitivity in females

Several families have been reported in which females show clinical features of NDI resembling the phenotype in males (for review [22•]). After the discovery of AQP2 gene mutations as a cause for autosomal recessive NDI, and in some cases for autosomal dominant NDI, it seemed that a satisfying explanation for the complete manifestation of the disease in some females had been found. However, four families were recently described in which symptomatic females do not have an AQP2 defect but are heterozygous for a mutation in the V_2 receptor [22•,43]. The maximal urine osmolality after DDAVP stimulation in these females did not exceed 200 mosmol/kg and an absence of extrarenal responses was found in two of the four women affected [22•]. One female patient showed a 50% increase in coagulation factor VIII after DDAVP administration [43]. Intriguingly, in three of the four families asymptomatic female family members carried the same V_2 receptor mutation as the manifesting females. The most likely explanation for the existence of different phenotypes in carriers of a V_2 receptor gene mutation, varying from no symptoms to complete manifestation of the disease, is skewed X-inactivation, which results in predominant expression of the mutant V_2 receptor allele. The discrepancy between the renal and extrarenal responses to DDAVP in one of the above patients may indicate differences in the X-inactivation pattern in distinct tissues.

Conclusion

The two genes involved in NDI encode proteins that reside at both ends of the cellular AVP-signalling cascade.

We are convinced that functional analysis of naturally occurring and targeted mutations in these genes will continue to be of indispensable value in determining the relationship between the structure and function of both the V_2 receptor and the AQP2 water channel. The discovery of different genetic causes of NDI has important implications for genetic counselling, especially in those families in which only one patient or a sole sibship is affected. In those cases a DDAVP test with measurement of coagulation and fibrinolytic parameters will help to discriminate between X-linked and non-X-linked NDI, at least in males. In females with a complete manifestation of NDI this test only indicates X-linked inheritance when there is a total absence of extrarenal responses.

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