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In-Depth Review

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Aquaporin-2: new mutations responsible for autosomal-recessive nephrogenic diabetes insipidus—update and epidemiology

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Abstract

It is clinically useful to distinguish between two types of hereditary nephrogenic diabetes insipidus (NDI): a 'pure' type characterized by loss of water only and a complex type characterized by loss of water and ions. Patients with congenital NDI bearing mutations in the vasopressin 2 receptor gene, *AVPR2*, or in the aquaporin-2 gene, *AQP2*, have a pure NDI phenotype with loss of water but normal conservation of sodium, potassium, chloride and calcium. Patients with hereditary hypokalemic salt-losing tubulopathies have a complex phenotype with loss of water and ions. They have polyhydramnios, hypercalciuria and hypo- or isosthenuria and were found to bear *KCNJ1* (ROMK) and *SLC12A1* (NKCC2) mutations. Patients with polyhydramnios, profound polyuria, hyponatremia, hypochloremia, metabolic alkalosis and sensorineural deafness were found to bear *BSND* mutations. These clinical phenotypes demonstrate the critical importance of the proteins ROMK, NKCC2 and Barttin to transfer NaCl in the medullary interstitium and thereby to generate, together with urea, a hypertonic milieu. This editorial describes two new developments: (i) the genomic information provided by the sequencing of the *AQP2* gene is key to the routine care of these patients, and, as in other genetic diseases, reduces health costs and provides psychological benefits to patients and families and (ii) the expression of *AQP2* mutants in Xenopus oocytes and in polarized renal tubular cells recapitulates the clinical phenotypes and reveals a continuum from severe loss of function with urinary osmolalities <150 mOsm/kg H2O to milder defects with urine osmolalities >200 mOsm/kg H2O.

Keywords: aquaporin-2 mutations; autosomal-dominant and -recessive nephrogenic diabetes insipidus; genetic testing; hereditary polyuric states

Identification of loss-of-function *AQP2* mutations responsible for autosomal-recessive or autosomal-dominant nephrogenic diabetes insipidus

Complex polyuric cases as described in the abstract are included in [1–6] and the benefit of genomic information in [7].

On the basis of 1-desamino-8-D-arginine vasopressin (dDAVP) infusion studies and measurements of plasma cyclic adenosine monophosphate (cAMP) levels following pharmacological intravenous doses of dDAVP, a vasopressin V2 synthetic analog, we first suggested that X-linked nephrogenic diabetes insipidus (NDI) was a pre-cyclic AMP defect [8, 9]. Male patients with X-linked NDI did not stimulate their coagulation factor release or plasma cyclic AMP level after a pharmacological infusion of dDAVP, a suggestion of a loss of function of both renal and extrarenal vasopressin V2 receptors. As in many other X-linked diseases, males are severely affected with polyuria and polydipsia, by contrast, women are rarely symptomatic (for a discussion on symptomatic heterozygous female patients bearing *AVPR2* mutations see: [10]). Subsequent haplotype analysis of X-linked NDI in ancestrally independent families followed by my laboratory revealed that all affected male subjects were segregated with X-q28 markers where the vasopressin receptor gene is localized [11]. At the same time, Birnbaumer [12] and her team cloned the vasopressin V2 receptor by expression, the readout signal was again stimulation of cAMP from transfected cells, and, in collaboration with her, we rapidly demonstrated that a frameshift mutation in the *AVPR2* gene, the gene coding for the vasopressin V2 receptor, was responsible for X-linked NDI [13].

Using dDAVP infusion studies and other families with severe polyuric characteristics in both male and female individuals, a non-X-linked form of NDI with a post-receptor (post-cAMP) defect was suggested [14–16]. 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 shown to be a

compound heterozygote for two missense mutations (R187C and S217P) in the *AQP2* gene [17]. Expression of each of these two mutations in Xenopus oocytes revealed non-functional water channels. The oocytes of the African clawed frog Xenopus have provided a most useful test bed for looking at the functioning of many channel proteins. (See recent characterization of proteins with gain of function responsible for autosomal-dominant pseudohypoaldosteronism type II [18].) Oocytes are large cells about to become mature eggs ready for fertilization. They have all the normal translation machinery of living cells, so they will respond to the injection of messenger RNA by making the encoded protein [19]. This convenient expression system was key to the discovery of AQP1 by Agre [20] because frog oocytes have very low permeability and survive even in freshwater ponds. A representation of the injection process and expression of two *AQP2* naturally occurring mutants responsible for autosomal-recessive NDI are described in Figure 1a. When subjected to a 20-mOsm osmotic shock, control oocytes have exceedingly low water permeability but test oocytes become highly permeable to water. These osmotic water permeability assays demonstrated an absence or very low water transport of the complementary RNA with *AQP2* mutations (Figure 1b). Immunofluorescence and immunoblot studies demonstrated that these recessive mutants were retained in the endoplasmic reticulum [21, 22].

Of interest, in the first identification of *AQP2* mutants by the Nijmegen group [17], the sequencing of the *AQP2* gene in this isolated patient with autosomal-recessive diabetes insipidus followed a candidate gene approach guided by new understanding of the necessity, after vasopressin recognition and signaling, to insert water channels in the luminal membrane of principal cells of the collecting ducts to achieve water reabsorption [23]. We used the new sequencing data provided by Deen *et al.* to solve the molecular identification of NDI in two inbred Pakistani girls with non-X-linked NDI originally reported by Langley *et al.* [16]. They were found to be homozygous for the *AQP2* V71M mutation, a recurrent mutation in Pakistani kindred since two other children from two other Pakistani families living in the UK, said to be unrelated, were found to bear the same mutation on the same *AQP2* haplotype (Figure 2).

To date, 46 putative disease-causing *AQP2* mutations have been identified in 52 NDI families (Table 1 and Figure 3). *AQP2* mutations in autosomal-recessive NDI, which are located throughout the gene, result in misfolded proteins that are retained in the endoplasmic reticulum

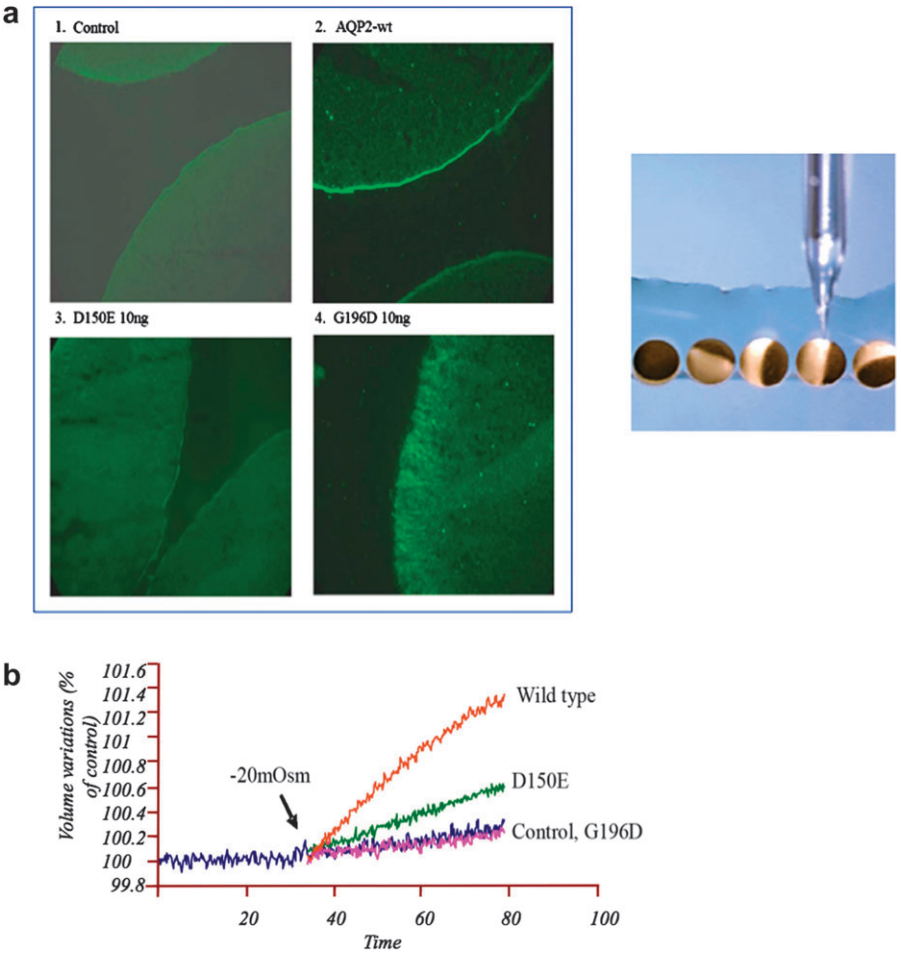

Fig. 1. (**a**) Immunofluorescence of *AQP2* expressed in oocytes. Oocytes were not injected (1 control) or injected with either AQP2-wt (2, 1 ng), AQP2-D150E (3, 10 ng) or AQP2-G196D (4, 10 ng) messenger RNAs and incubated for 3 days prior to assay. Oocytes were immunostained and visualized with antibodies to AQP2. The injection process is represented in the right of the figure [21]. (**b**) Determination of water permeabilities (Pf) of wild-type (WT) AQP2 and mutants expressed in Xenopus oocytes. Oocytes were injected with either AQP2-wt (1 ng), AQP2-D150E (10 ng) or AQP2-G196D (10 ng) messenger RNAs and incubated for 2 days prior to assay. Determination of water permeabilities was performed by evaluation of volume increase in oocytes as induced by a 20-mosmol/kg H2O hypotonic shock [21].

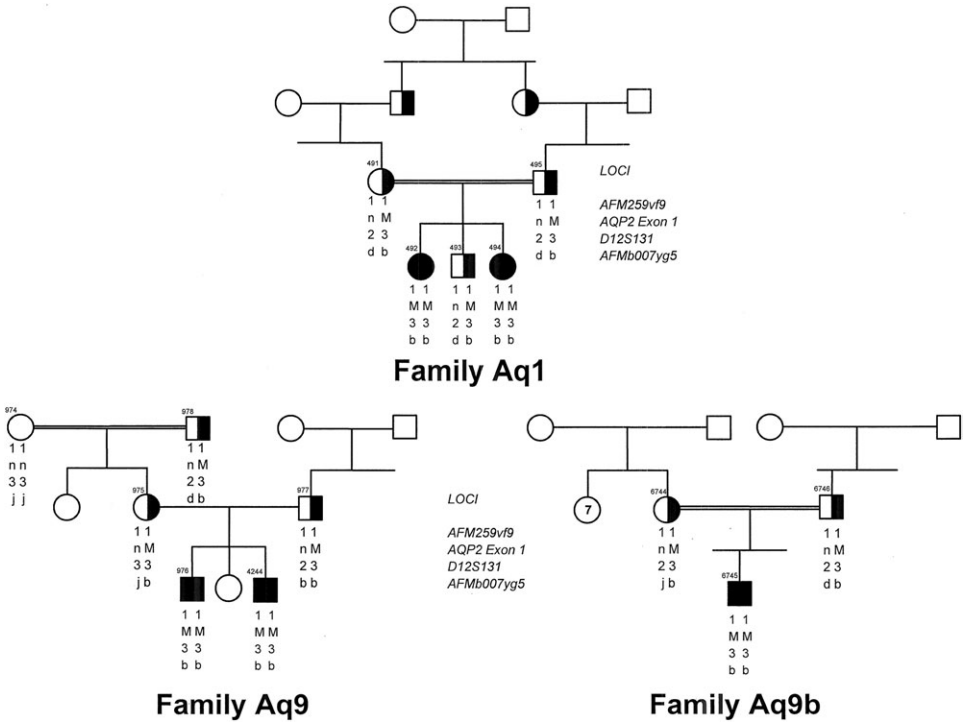

Fig. 2. Pedigree of three Pakistani families referred to our laboratory each bearing the V71M AQP2 mutation (M). Family Aqp1 was first described by Langley [16]. Squares and circles represent male and female subjects, respectively, with unaffected individuals (open symbols), carriers (half-filled symbols) and affected individuals (solid symbol); n indicates normal allele. Haplotypes consist of markers that flank the AQP2 gene and that have been described previously [24]. The alleles bearing the individual mutations are identical suggesting a common ancestry.

(Table 1; Figure 3). In contrast, the dominant mutations reported to date are located in the region that codes for the carboxyl terminus of *AQP2* [55]. Dominant AQP2 mutants form heterotetramers with wt-AQP2 and are misrouted [28, 36, 40, 45–48, 51–54]. Patients bearing these dominant mutations have a less severe phenotype as compared to patients who are compound heterozygotes or homozygotes for recessive mutations: the patient and her daughter first described to bear the AQP2-E258K-dominant mutation increased their urine osmolality to 350 mOsm/kg H_2O following dDAVP [45]. Also the patient with a detailed phenotype described by Robertson and Koop [25] increased her urine osmolality to 220 mOsm/kg H_2O during a mildly hypertonic dehydration, to 258 mOsm/kg H_2O after dDAVP and to 305 mOsm/kg H_2O after hydrochlorothiazide and indomethacin. This patient was found to be heterozygous for the R254Q mutation, possibly interfering with the S256 phosphorylation site [40]. In the mutant AQP2 (763–772) knockin mice, Sohara *et al.* [56] demonstrated a slight increase in urine osmolality following dehydration but a marked increase after the administration of Rolipram, a phosphodiesterase-4 inhibitor.

In patients with hereditary polyuria, an X-linked inheritance will suggest X-linked *AVPR2* mutations and autosomal-recessive cases will be in favor of *AQP2* mutations but we are recommending the sequencing analysis of *AVPR2* first and then *AQP2* genes in all patients. These genes are, with a few exceptions, relatively small and easy to sequence. This genomic information is key to the routine care of patients with congenital polyuria and, as in other genetic diseases, reduces health costs and provides psychological benefits to patients and their families: an example of the possibility to help families living at a distance from a referral center is given in Figure 4.

Signaling of vasopressin and insertion of AQP2 water channels in the luminal membrane of principal cells of the collecting duct: the work of five Nobel Prize recipients

The transfer of water across the principal cells of the collecting ducts is now known at such a detailed level that billions of molecules of water traversing the membrane can be represented; see useful teaching tools at http://www.mpibpc.gwdg.de/abteilungen/073/gallery.html and http://www.ks.uiuc.edu/research/aquaporins. The 2003 Nobel Prize in chemistry was awarded to Peter Agre and Roderick MacKinnon, who solved two complementary problems presented by the cell membrane: how does a cell let one type of ion through the lipid membrane to the exclusion of other ions? And how does it permeate water without ions? This contributed to creating momentum and renewed interest in basic discoveries related to the transport of water and indirectly to diabetes insipidus [57, 58]. The first step in the action of AVP (Synthetized by du Vigneaud, Nobel Prize in Chemistry 1955) [59] on water excretion is its binding to arginine vasopressin type 2 receptors (hereafter referred to as V2 receptors) on the basolateral membrane of the collecting duct cells (Figure 5). The human *AVPR2* gene that codes for the V2 receptor is located in the chromosome region Xq28 and has three exons and two small introns [12, 60]. The sequence of the complementary DNA predicts a polypeptide of 371 amino acids with seven

Table 1. Listing of 46 putative disease-causing AQP2 mutations

Count	No. of families	Name of mutation	Domain	Nucleotide change	Predicted consequence	Reference
		Missense [25]				
1	1	M1I	NH2	ATG-to-ATT	Met-to-Ile	Sahakitrungruang *et al.* [26]
2	1	L22V	TMI	CTC-to-GTC	Leu-to-Val	Canfield *et al.* [27]
3	1	V24A	TMI		Val-to-Ala	Leduc-Nadeau *et al.* [22]
4	1	L28P	TMI	CTC-to-CCC	Leu-to-Pro	Marr *et al.* [28]
5	1	G29S	TM1		Gly-to-Ser	Sahakitrungruang *et al.* [26]
6	2	A47V	TMII	GCG-to-GTG	Ala-to-Val	Marr *et al.* [28]; Muller *et al.* [29]
7	2	Q57P	TMII	CAG-to-CCG	Glu-to-Pro	Lin *et al.* [24]
8	1	G64R	CII	GGG-to-AGG	Gly-to-Arg	van Lieburg *et al.* [30]
9	1	N68S	CII	AAC-to-AGC	Asn-to-Ser	Mulders *et al.* [31]
10	1	A70D	CII	GCC-to-GAC	Ala-to-Asp	Cheong *et al.* [32]
11	2	V71M	CII	GTG-to-ATG	Val-to-Met	Marr *et al.* [28]
12	2	G100V	TMIII	GGA-to-GTA	Gly-to-Val	Lin *et al.* [24]
13	1	G100R	TMIII	GGA-to-AGA	Gly-to-Arg	Carroll *et al.* [33]
14	1	I107D	EII	ATC-to-AAC	Ile-to-Asp	Zaki *et al.* [34]
15	1	T125M	EII	ACG-to-ATG	Thr-to-Met	Goji *et al.* [35]; Kuwahara [36] (same family)
16	1	T126M	EII	ACG-to-ATG	Thr-to-Met	Mulders *et al.* [31]
17	1	A147T	TMIV	GCC-to-ACC	Ala-to-Thr	Mulders *et al.* [31]
18	2	D150E	ICII	GAT-to-GAA	Asp-to-Glu	Guyon *et al.* [21]; Iolascon *et al.* [37]
19	1	V168M	TMV	GTG-to-ATG	Val-to-Met	Vargas-Poussou *et al.* [38]
20	1	G175R	TMV	GGG-to-AGG	Gly-to-Arg	Goji *et al.* [35]; Kuwahara [36] (same family); Boccalandro *et al.* [39]
21	1	G180S	EIII	GGC-to-AGC	Gly-to-Ser	Carroll *et al.* [33]
22	1	C181W	EIII	TGC-to-TGG	Cys-to-Trp	Canfield *et al.* [27]
23	1	P185A	EIII	CCT-to-GCT	Pro-to-Ala	Marr *et al.* [28]
24	3	R187C	EIII	CGC-to-TGC	Arg-to-Cys	Deen *et al.* [17]; van Lieburg *et al.* [30]; de Mattia *et al.* [40]; Leduc-Nadeau *et al.* [22]
25	1	R187H	EIII	CGC-to-CAC	Arg-to-His	Cheong 2005 #1846 [32]
26	1	A190T	EIII	GCT-to-ACT	Ala-to-Thr	Kuwahara [36]; de Mattia *et al.* [40]
27	1	G196D	EIII	GGC-to-GAC	Gly-to-Asp	Guyon *et al.* [21]
28	1	W202C	EIII	TGG-to-TGT	Trp-to-Cys	Oksche *et al.* [41]
29	1	G215C	TMVI	GGC-to-TGC	Gly-to-Cys	Iolascon *et al.* [37]
30	2	S216P	TMVI	TCC-to-CCC	Ser-to-Pro	Deen *et al.* [17]; Vargas-Poussou *et al.* [38]
31	1	S216F	TMVI	TCC-to-TTC	Ser-to-Phe	Moon *et al.* [42]
32	1	K228E	CIV		Lys-to-Glu	Leduc-Nadeau *et al.* [22]
33	1	R254Q	CIV	CGG-to-CAG	Arg-to-Gln	Robertson *et al.* [25]; Savelkoul *et al.* [43]
34	1	R254L	CIV	CGG-to-CTG	Arg-to-Leu	de Mattia *et al.* [40]; de Mattia *et al.* [44]
35	1	E258K	CIV	GAG-to-AAG	Glu-to-Lys	Mulders *et al.* [45]; Kamsteeg *et al.* [46]; Kamsteeg *et al.* [47]; Kamsteeg *et al.* [48]
36	2	P262L	CIV	CCG-to-CTG	Pro-to-Leu	Kuwahara [36]; de Mattia *et al.* [40]
		Nonsense [2]				
1	2	R85X	CII	CGA-to-TGA	Arg-to-stop	Vargas-Poussou *et al.* [38]; Bircan *et al.* [49]
2	1	G100X	TMIII	GGA-to-TGA	Gly-to-stop	Hochberg *et al.* [50]
		Frameshift [6]				
1	1	369delC	EII	1bp deletion	Stop at Codon 131	van Lieburg *et al.* [30]
2	1	721delG	CIV	1 bp deletion	Post-elongation	Kuwahara *et al.* [51]; Ohzeki *et al.* [52]
3	1	727delG	CIV	1 bp deletion	Post-elongation	Marr *et al.* [53]
4	1	763-772del	CIV	10 bp deletion	Post-elongation	Kuwahara *et al.* [51]
5	1	779–780insA	CIV	1 bp insertion	Post-elongation	Kamsteeg *et al.* [54]
6.	1	812-818del	CIV	7 bp deletion	Post-elongation	Kuwahara *et al.* [51]
		Splice-site [2]				
1	1	IVS2-1G>A	NA	G-to-A	NA	D. G. Bichet *et al.*, unpublished
2	1	IVS3+1G>A	NA	G-to-A	NA	Marr *et al.* [53]
46	53	Note: a family was counted only once if the affected child was homozygous or if the affected child was compound heterozygous				

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) (1994 Nobel Prize in Physiology and Medicine to Rodbell and Gilman for signal transduction and G-proteins) and promotes the cAMP-mediated incorporation of water channels into the luminal surface of these cells [61]. E. Sutherland and T. Rall isolated cAMP in 1956 and Sutherland was awarded the Nobel Prize in Physiology or Medicine in 1971 [62]. There are two ubiquitously expressed intracellular cAMP receptors: (i) the classical protein kinase A (PKA) that is a cAMP-dependent protein kinase and (ii) the recently discovered exchange protein directly activated by cAMP that is a cAMP-regulated guanine nucleotide exchange factor. Both these receptors contain an evolutionally conserved cAMP-binding domain that acts as a molecular switch for sensing intracellular cAMP levels to control diverse biological functions [63]. Several proteins participating in the control of cAMP-dependent AQP2 trafficking have been identified; for example, A-kinase-anchoring proteins tethering PKA to cellular compartments; phosphodiesterases regulating the local cAMP level; cytoskeletal components such as F-actin and microtubules; small GTPases of the Rho family controlling cytoskeletal dynamics; motor proteins transporting AQP2-bearing vesicles to and from the plasma membrane for exocytic insertion and endocytic retrieval and SNAREs inducing membrane fusions, hsc70, a chaperone

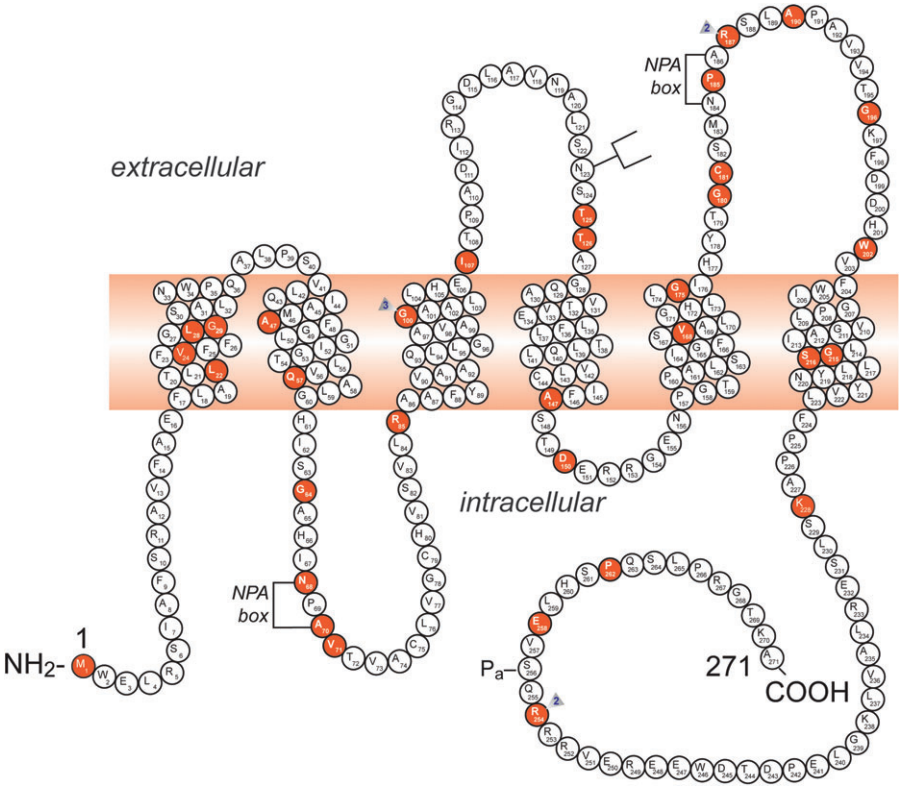

Fig. 3. A representation of the AQP2 protein and identification of 46 putative disease-causing AQP2 mutations. A monomer is represented with six transmembrane helices. The location of the PKA phosphorylation site (Pa) is indicated. The extracellular, transmembrane and cytoplasmic domains are defined according to Deen *et al.* [17]. Solid symbols indicate the location of the mutations (for references, view Table 1): M1I; L22V; V24A; L28P; G29S; A47V; Q57P; G64R; N68S; A70D; V71M; R85X; G100X; G100V; G100R; I107D; 369delC; T125M; T126M; A147T; D150E; V168M; G175R; G180S; C181W; P185A; R187C; R187H; A190T; G196D; W202C; G215C; S216P; S216F; K228E; R254Q; R254L; E258K and P262L. GenBank accession numbers—AQP2: AF147092, Exon 1; AF147093, Exons 2 through 4. NPA motifs and the *N*-glycosylation site are also indicated.

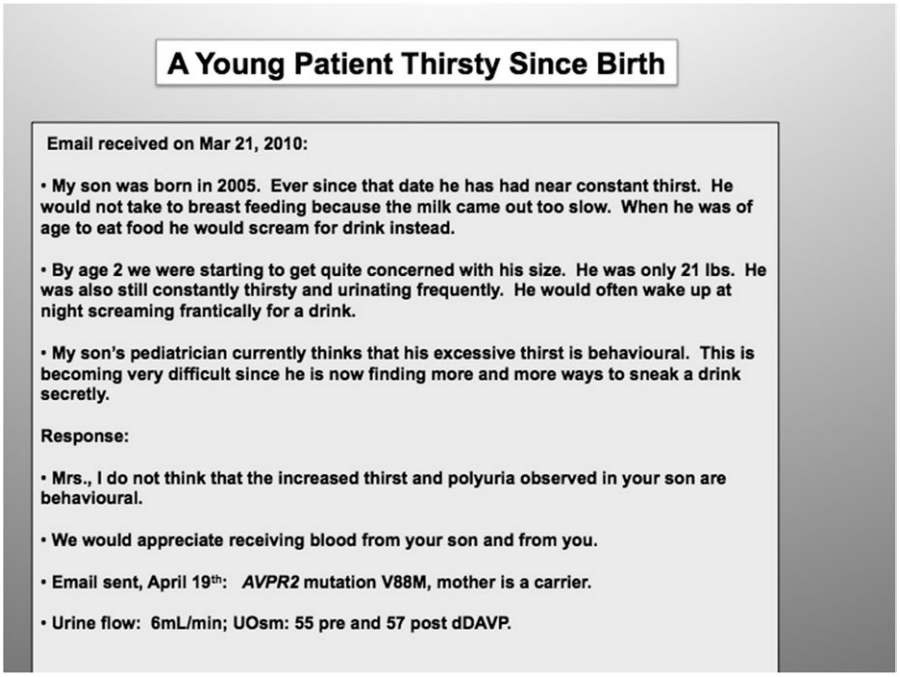

Fig. 4. A young patient's thirsty since birth.

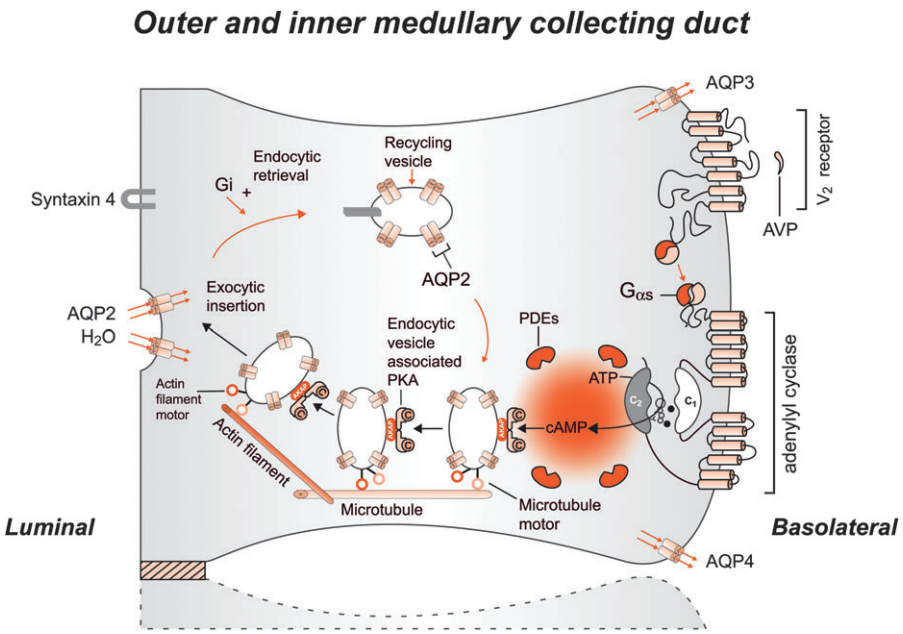

Fig. 5. Schematic representation of the effect of vasopressin (AVP) to increase water permeability in the principal cells of the collecting duct. AVP is bound to the V_2 receptor (a G-protein-linked receptor) on the basolateral membrane. The basic process of G-protein-coupled receptor signaling consists of three steps: a hepta-helical receptor that detects a ligand (in this case, AVP) in the extracellular milieu, a G-protein ($G_{\alpha s}$) that dissociates into a subunits bound to guanosine triphosphate and bg subunits after interaction with the ligand-bound receptor and an effector (in this case, adenylyl cyclase) that interacts with dissociated G-protein subunits to generate small molecule second messengers. AVP activates adenylyl cyclase, increasing the intracellular concentration of cAMP. The topology of adenylyl cyclase is characterized by two tandem repeats of six hydrophobic transmembrane domains separated by a large cytoplasmic loop and terminates in a large intracellular tail. The dimeric structure (C_1 and C_2) of the catalytic domains is represented. Conversion of adenosine triphosphate (ATP) to cAMP takes place at the dimer interface. Two aspartate residues (in C_1) coordinate two metal co-factors (Mg^{2+} or Mn^{2+} represented here as two small black circles), which enable the catalytic function of the enzyme. Adenosine is shown as an open circle and the three phosphate groups (ATP) are shown as smaller open circles. PKA is the target of the generated cAMP. The binding of cAMP to the regulatory subunits of PKA induces a conformational change, causing these subunits to dissociate from the catalytic subunits. These activated subunits (C) as shown here are anchored to an aquaporin-2 (AQP2)-containing endocytic vesicle via an A-kinase-anchoring protein. The local concentration and distribution of the cAMP gradient are limited by phosphodiesterases (PDEs). Cytoplasmic vesicles carrying the water channels (represented as homotetrameric complexes) are fused to the luminal membrane in response to AVP, thereby increasing the water permeability of this membrane. The dissociation of the A-kinase-anchoring protein from the endocytic vesicle is not represented. Microtubules and actin filaments are necessary for vesicle movement toward the membrane. When AVP is not available, AQP2 water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. Aquaporin-3 (AQP3) and aquaporin-4 (AQP4) water channels are expressed constitutively at the basolateral membrane.

important for endocytic retrieval. These processes are the molecular basis of the vasopressin-induced increase in the osmotic water permeability of the apical membrane of the collecting tubule [64–66].

How could we decrease urine flow in patients bearing AQP2 mutations?

The actions of vasopressin in the distal nephron are possibly modulated by prostaglandin E2, nitric oxide [67] and by luminal calcium concentration. PGE2 is synthesized and released in the collecting duct, which expresses all four E-prostanoid receptors (EP1–4). Both EP2 and EP4 can signal via increased cAMP. Olesen *et al.* [68] hypothesized that selective EP receptor stimulation could mimic the effects of vasopressin and demonstrated that, at physiological levels, PGE2 markedly increased apical membrane abundance and phosphorylation of AQP2 *in vitro* and *ex vivo*, leading to increased cell water permeability. In their experiments, both EP2- and EP4-selective agonists were able to mimic these effects. Furthermore, an EP2 agonist was able to positively regulate urinary-concentrating mechanisms in an animal model of NDI where AVPR2 receptors were blocked by Tolvaptan, a non-peptide V2 antagonist. These results reveal an alternative mechanism for regulating water transport in the collecting duct that have major importance for understanding whole-body water homeostasis and provide a rationale for investigations into EP receptor agonist use in X-linked NDI treatment.

In patients with *AQP2* mutations, bypassing the vasopressin V2 receptor will not be of any therapeutic value; therefore, the classical decrease in osmotic load described by Orloff and Earley [69], well before any molecular identification of the genes responsible for hereditary NDI, should be used. When the urine osmolality is fixed, as in nephrogenic DI, the urine output is determined by solute excretion. Suppose that the maximum urine osmolality is 150 mosmol/kg. In this setting, the daily urine volume will be 5 L if solute excretion is in the normal range at 750 mosmol/day, but only 3 L if solute excretion is lowered to 450 mosmol/day by dietary modification.

These observations provide the rationale for the use of a low-salt low-protein diet to diminish the urine output in NDI. The reduction in urine output will be directly proportional to the decrease in solute intake and excretion. Restriction of salt intake to $\leq$100 mEq/day (2.3 g sodium) and protein intake to $\leq$1.0 g/kg may be reasonable goals, but such diets are not easy to achieve and maintain. Furthermore, protein restriction in infants and young children may be harmful and is not advised. A thiazide diuretic (such as hydrochlorothiazide, 25 mg once or twice daily) acts by inducing mild volume depletion. As little as a 1–1.5 kg weight loss in adults with hereditary, NDI can reduce the

urine output by >50% [e.g. from 10 to <3.5 L/day in a study of patients with NDI on a severely sodium-restricted diet (9 mEq/day)] [69, 70].

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Conflict of interest statement. None declared.

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