Characterization of an Aquaporin-2 Water Channel Gene Mutation Causing Partial Nephrogenic Diabetes Insipidus in a Mexican Family: Evidence of Increased Frequency of the Mutation in the Town of Origin

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Abstract. A Mexican family with partial congenital nephrogenic diabetes insipidus (NDI) that resulted from a mutation in the aquaporin-2 water channel (*AQP2*) was characterized, and the source of this rare mutation was traced to the family's town of origin in Mexico. Affected individuals with profound polyuria and polydipsia were homozygous for an autosomal recessive missense V168M mutation in the *AQP2* gene. Expression in oocytes revealed that, although retained in the endoplasmic reticulum (ER) to a great extent, a considerable amount of the partially functional AQP2-V168M was expressed at the plasma membrane, and that its ER retention was less than AQP2-T126M, a functional mutant in severe recessive NDI. None of the affected AQP2-V168M individuals had neurologic deficits, which also suggested a milder form of the disease. The homozygous individuals reported subjective improvement in polyuria and polydipsia with the use of dDAVP (1-desamino-8-D-arginine-vasopressin). When clinically tested, infusion of dDAVP at variable doses produced a partial increase in the urinary osmolality in homozygous individuals and decreased their water intake. Heterozygotes were unaffected when compared with controls. Samples were obtained from the population of the Mexican town of origin of the family; 30% of the population was heterozygous for the V168M *AQP2* mutation and 1% was homozygous for the mutation. The high frequency of this rare mutation in the town provides evidence for an important health care problem in the village with consequences for future generations.

Diabetes insipidus is defined by a 24-h urine volume greater than 3 L and urinary osmolality less than 300 mOsm/kg (1). Diabetes insipidus can be of central or nephrogenic origin, and it can be acquired or hereditary. Individuals affected with nephrogenic diabetes insipidus (NDI) are prone to severe dehydration if unable to access liquids and can experience failure to thrive, mental retardation, and death early in life (2–5). Adequate intake of water allows a normal lifespan and good physical and mental development (6).

Inherited NDI results from mutations in two different genes; 90% occur in the gene that codes for the vasopressin 2 receptor (*AVPR2*) found on the X chromosome (7) and 10% in the gene for aquaporin-2 (*AQP2*) (8). The aquaporin membrane pore is located in the collecting duct cells and is the primary target by which vasopressin regulates permeability of water. *AQP2* (xm_006751) is located on chromosome 12q13, consists of four exons and encodes a 271–amino acid protein with six transmembrane domains. Thirty mutations in *AQP2* have been reported, most commonly recessive missense mutations that cause misfolding of the protein and retention in the endoplasmic reticulum (ER) (3,4,9–16). Dominant mutations are less common and are characterized by retention of AQP2 in the Golgi complex region, late endosomes/lysosomes, or basolateral membrane due to the formation of heterotetramers of normal and abnormal AQP2 (17–21).

We have identified a family from Mexico with familial NDI caused by a recessive mutation in the *AQP2* gene. The mutant aquaporin-2 is partially functional in *Xenopus* oocytes. Affected family members reported improvement in thirst and urination with the use of nasal and oral dDAVP (1-desamino-8-D-arginine-vasopressin), prompting the study of the clinical differences between homozygous, heterozygous and controls. Finally, a genetic epidemiologic study was performed in the town of origin of the family in Mexico.

Materials and Methods

Water Deprivation Tests

The study was approved by the Institutional Review Board of the University of Texas Health Science Center at Houston. Written informed consent was obtained from the family members that participated in the genetic and clinical studies, and verbal informed consent was obtained from the townspeople in the genetic epidemiologic study done in Mexico. Clinical studies were done at the Clinical Research Center of The University of Texas Health Science Center at Houston.

dDAVP was stopped 24 h before any testing. Baseline 24-h urine collections were performed to measure volume and urine osmolality. The technique used for the water deprivation test has been previously described (22,23). Baseline samples for urine osmolality (Uosm), serum osmolality (Sosm), serum electrolytes, and plasma arginine vasopressin (AVP) were taken. AVP samples were collected in heparinized plasma and immediately frozen for future processing. Hypertonicity was initiated with a continuous 3% saline infusion at a rate of 0.06 ml/kg/min. Sosm was checked hourly until a level of 300 mOsm/kg was reached. Then, a second plasma sample for AVP was obtained before 1 μg of dDAVP was provided intravenously to each subject. Urine volume, Uosm, and Sosm were monitored hourly for the next 2 h.

dDAVP Challenge Test

Controls and homozygous and heterozygous family members underwent a dDAVP challenge test with water restriction as a surrogate for a full water deprivation test (24–27). Fluid intake was limited to 250 ml/h. Baseline samples for Uosm, Sosm, serum electrolytes, blood urea nitrogen, glucose, creatinine, and calcium were obtained. Urinary volume and fluid intake were monitored hourly. Uosm was checked 3 h after each dose of dDAVP to ascertain maximal effect of the drug (24–28). No further doses of dDAVP were provided when Uosm was above 800 mOsm/kg (22,24,29). All of the dDAVP doses were provided intravenously. The first dose of dDAVP consisted in 1 μg for controls and heterozygotes and 5 μg for homozygotes. Three hours later, those with Uosm below 800 mOsm/kg received a second dose of dDAVP, 4 μg for the heterozygotes and controls and 0.4 μg/kg for the homozygotes. Uosm was checked 3 h later.

Mutational Analysis

Genomic DNA from the family members was isolated from peripheral blood samples in adults and from buccal samples in children. The DNA was extracted with the Pure Gene genomic DNA isolation kit (Gentra Systems). Intron-based primers were used to PCR-amplify the four exons of *AQP2* (10). PCR amplifications were carried out with HotstarTaq DNA polymerase (Qiagen, Valencia, CA) in a two-cycle amplification program to amplify the genomic DNA. The DNA fragments were sequenced with ABI Big Dye terminator kit on an ABI Prism 310 DNA Analyzer (Applied Biosystems, Foster City, CA).

Generation of the Mutant AQP2 cDNA Construct

PCR techniques were used to introduce a G502A transversion in human *AQP2* cDNA, which was cloned into the plasmid pT7Ts-AQP2. The forward primer, TGCCATGGCGTTTGGCTTGG, has a *Nco*I restriction site at the 5' end. The reverse primer, 5'-ATGGATCCCAAGGAGGTGGCCCAGGGCCA**T**AGAGAAGCC-3' has a *Bam*HI site at the 5' end. A G502A transition was introduced into the middle of the reverse primer. PCR was performed by using the plasmid pT7Ts-AQP2 as the template. The PCR product was digested by *Nco*I and *Bam*HI and inserted into the corresponding sites of

pT7Ts-AQP2. The G502A mutation of *AQP2* was confirmed by sequencing.

Oocyte Experiments

Linearization of the pT7Ts-AQP2 and pT7Ts-AQP2-V168M constructs, cRNA synthesis, preparation and injection of oocytes, cell swelling assays, immunoblotting, and immunocytochemistry were performed as described (30). The obtained water permeabilities were expressed as means $\pm$ SEM. Differences between groups were tested by t test corrected by the Bonferroni multiple-comparisons procedure. Differences were considered statistically significant for $P < 0.05$. To determine the functionality of AQP2 mutants in relation to wt-AQP2, all immunoblot signals of AQP2 proteins in the plasma membrane of oocytes were semiquantified by densitometric scanning and compared with the signals of a twofold dilution series of wt-AQP2, which was blotted in parallel.

Because the quality of cRNA preparations and injected amounts show variation for different cRNAs, the expressed amounts of the AQP2 proteins from determined cRNA amounts is not reliable. Therefore, our analyses with AQP2-V168M focused on whether AQP2-V168M is a functional water channel and if so, to what extent compared with wt-AQP2. Also, we wanted to determine whether it is retained the ER, and if so, whether its retention is different from wt-AQP2 and from AQP2-T126M, another AQP2 mutant that confers severe instead of partial NDI in patients. For these analyses, similar protein expression levels are needed, which is much better controlled in oocytes than in mammalian cells. For determination whether a mutant is retained in the cell, the plasma membrane expression levels have to be compared for those oocytes that show similar total membrane expression levels because the level of plasma membrane expression goes up with increased expression levels.

In a second analysis, by comparing the water permeabilities conferred by similar plasma membrane expression levels of different aquaporins, one can estimate the single channel permeability. For this, the different amounts of wt-AQP2 expressed in the plasma membrane fraction of oocytes and the corresponding water permeability (P_f) values were fitted to the exponential function $y = a[1 - \exp(-bx)] + c$, where y is P_f and x is the amount of protein in arbitrary units. The amount of wt-AQP2 that would be necessary to obtain the P_f value observed for the mutants was calculated from the equation. The ratio of the amount of wt-AQP2 and mutant AQP2 for the P_f that had been obtained for the mutant indicates the single channel water permeability of the mutant in relation to wt-AQP2 in percentage.

Genetic Epidemiologic Data

Samples were collected from individuals in a town in Mexico. Subjects were asked for voluntary participation at their homes or in public areas with the aid of the local physician and nurse. Participants were over 18 yr old, born in the town, and had at least one parent native to the town. Only one sibling of each family was sampled. Buccal cell samples were collected with the Scope mouthwash technique (31,32). The samples had no personal identifiers and were stored at 4°C until processed. DNA was extracted and *AQP2* mutation in exon 2 was analyzed by direct sequencing. The genotype frequencies observed were compared with those expected under the assumption of random mating; a χ^2 test was used for deviation from Hardy-Weinberg equilibrium.
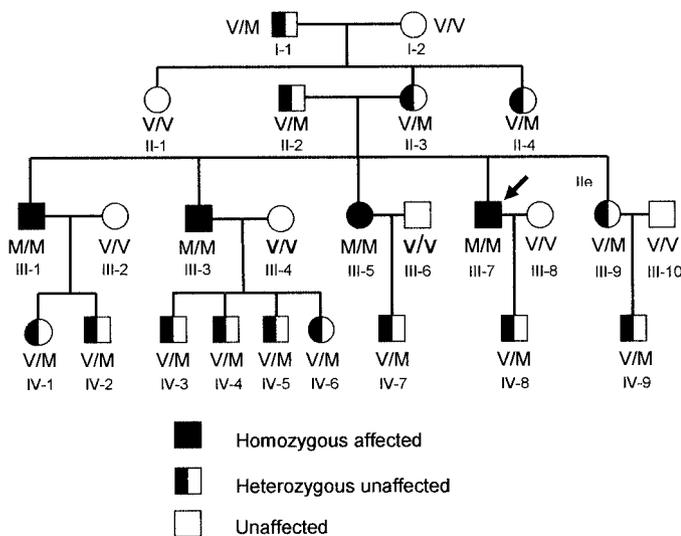
Statistical Analyses

Comparison between water deprivation and dDAVP tests, age groups in the population samples, and the percentage of permeability

of the mutant relative to wt-AQP2 was determined by *t* test (33). Deviation from the Hardy-Weinberg equilibrium was evaluated with the bootstrapping and permutation techniques available in the genetics library as programmed in R (34).

Results

Clinical Description of the Mexican Family

The family studied was originally from a small town northeast of Monterrey, Mexico (Figure 1). Both parents were born in the same town but denied consanguinity. The town started as a ranch founded in 1600s and consists of a larger central area and six satellite communities that share the same ancestry. The total population of the town was 2078, according to the Mexican census from the year 2000 (35). Both parents of the studied family had normal thirst and urination and were healthy except for moderate hypertension in the father. The mother had experienced no miscarriages, and none of her children had died. The first four of their five children had polydipsia and polyuria soon after birth, and all had normal physical and mental development without other medical problems. At the age of 20 yr, an affected sibling (III-5) had a severe hypernatremic episode during a cesarean section. After recovering from the episode, she was treated with dDAVP and reported a 50% decrease in nocturnal thirst and urination. The index patient, the fourth child of the family, approached the endocrinology clinic at the University of Texas Houston and was the first one to be studied by our group. He worked as a construction worker in the Houston area and was able to work in hot climates without experiencing dehydration by drinking large amounts of water. He also reported a decrease in nocturnal polydipsia and polyuria by 50% when treated with dDAVP.

Mutational Analysis

DNA from the proband was used to sequence the exons and flanking intronic sequences of *AQP2*. A homozygous G to A nucleotide alteration was found at the position 502 in exon 2, leading to a valine to methionine alteration at amino acid 168 in the fifth transmembrane domain of the aquaporin-2 water channel. Exon 2 was sequenced using DNA from all family members. All of the affected family members were homozygous for the mutation, and all other sequenced family members were heterozygous, except the maternal grandmother who did not carry the mutation (Figure 1). Analysis of DNA from 31 white and 29 Hispanic individuals obtained from the Houston area failed to reveal the mutation. A C/T polymorphism immediately adjacent to the mutation (position 501 in exon 2) was identified in the Hispanic controls. The polymorphism did not alter an amino acid, and the frequency of the polymorphism was 14%.

Functional Analysis of AQP2-V168M

To determine the biologic basis for NDI in this family, the V168M encoding mutation was introduced in the human *AQP2* cDNA contained in an oocyte expression vector, the cRNA was transcribed and then injected into oocytes. cRNA coding for AQP2-T126M, a functional mutant in recessive NDI (14), was taken along. Determination of the water permeability (P_f) of noninjected control and cRNA-injected oocytes revealed that oocytes expressing AQP2-V168M had a significantly higher P_f than control oocytes, indicating that AQP2-V168M is a functional water channel (Figure 2). At the dosages used here, AQP2-T126M did not confer any water permeability, but at high expression levels, AQP2-T126M has shown to be a functional water channel (14,36,37).

Because the quality of produced cRNA and the injected amounts can vary, total membranes of these oocytes were immunoblotted for AQP2 to reveal their expression levels (Figure 3). At moderate expression levels, AQP2-V168M and

Figure 1. Pedigree of the Mexican family. Darkened symbols indicate individuals with nephrogenic diabetes insipidus (NDI) and the proband is indicated with an arrow. The amino acid alteration in the aquaporin-2 (AQP2) protein is indicated below the symbol. V, valine (wild-type sequence); M, methionine (mutant sequence). Individuals marked M/M were affected, and individuals marked V/V or V/M (heterzygotes) were unaffected.
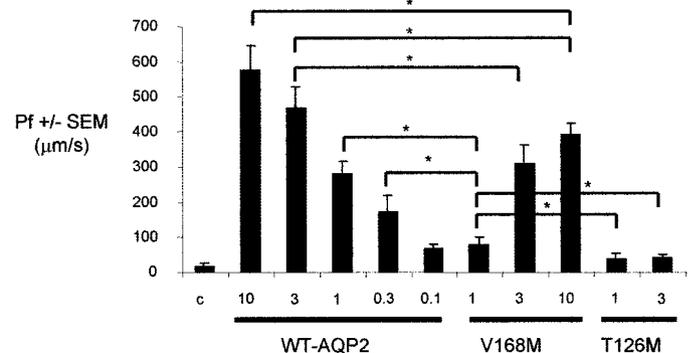

Figure 2. Osmotic water permeability of oocytes expressing aquaporin-2 (AQP2)–V168M. Oocytes were not injected (c) or were injected with the indicated amounts (ng) of cRNAs encoding wild-type AQP2 (WT-AQP2), AQP2-V168M, or AQP2-T126M. Two days after injection, the mean water permeabilities ($P_f \pm$ SEM in μm/s; *n* = 12) were determined in a standard swelling assay. Significant differences between samples ($P < 0.05$) are indicated by asterisks.
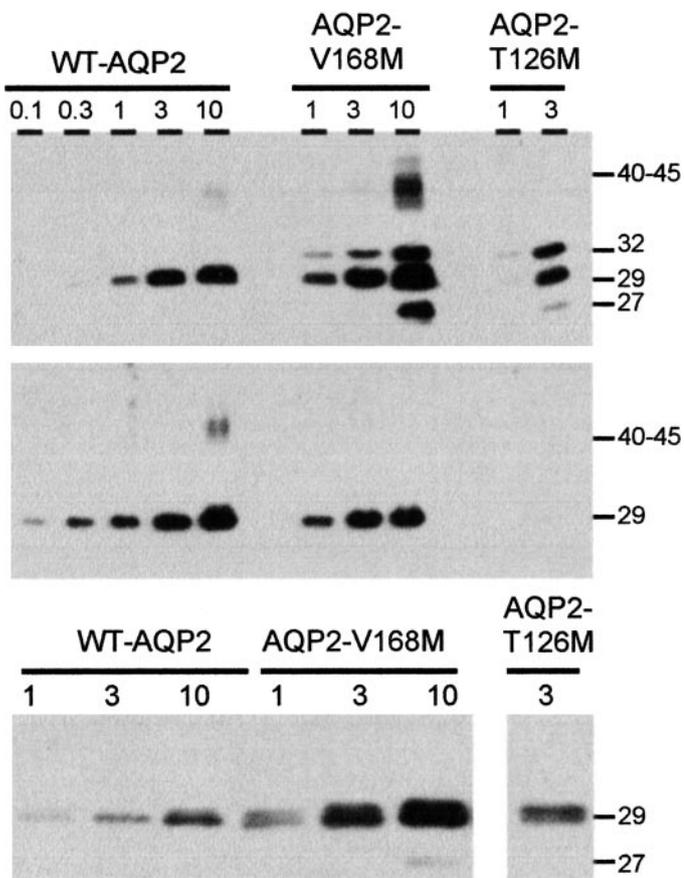
Figure 3. Immunoblot analysis of aquaporin-2 (AQP2)–V168M expressed in oocytes. From 12 oocytes, injected as described in Figure 2, total membranes (top) or plasma membranes (middle) were isolated and immunoblotted for AQP2. Total membrane samples were also immunoblotted for AQP2 after removal of all sugar moieties with endoglycosidase F (bottom). The masses of unglycosylated (29 kD), high-mannose glycosylated (32 kD), complex-glycosylated (40 to 45 kD), and a degradation product (27 kD) are indicated.

AQP2-T126M were expressed as 29 kD nonglycosylated and 32 kD high-mannose glycosylated proteins (Figure 3, upper panel), which is characteristic for ER-retained AQP2 proteins (30), whereas wt-AQP2 was only detected as a 29-kD band. At higher expression levels (10 ng cRNA), however, complex-glycosylated (40 to 45 kD; wt-AQP2 and AQP2-V168M) and a 27-kD degradation product (AQP2-V168M, AQP2-T126M) were detected as well.

Immunoblotting of plasma membranes of these oocytes revealed a clear plasma membrane expression for wt-AQP2 and AQP2-V168M at every injected amount, whereas AQP2-T126M was not detected in the plasma membranes (Figure 3, middle panel). To relate the plasma membrane and total membrane expression levels, total membranes equivalents of 1-, 3-, and 10-ng injections were treated with endoglycosidase F, which removes all sugar moieties, and immunoblotted for AQP2 (Figure 3, lower panel). The immunoblot signals were densitometrically scanned and semiquantified by comparison with the immunoblot signals obtained from a twofold dilution series of wt-AQP2 (data not shown). As reported, a decreased

ratio of plasma membrane *versus* total membrane expression for an AQP2 mutant compared with that of wt-AQP2 indicates that the mutant protein is retained within the cell (38).

As can be seen in Figure 3, AQP2-V168M showed a reduced plasma membrane *versus* total membrane ratio than wt-AQP2 (*e.g.,* compare signals of 3 ng AQP2-V168M to 10 ng wt-AQP2). Densitometric analysis revealed a fourfold reduced plasma membrane expression of AQP2-V168M compared with wt-AQP2. Compared with AQP2-T126M, however, the trafficking of AQP2-V168M to the plasma membrane was much better because the total membrane expression level from 3 ng AQP2-T126M cRNA was in between those of 1 and 3 ng AQP2-V168M (Figure 3, bottom), whereas no plasma membrane expression was detected for AQP2-T126M, in contrast to that of oocytes injected with 1 ng AQP2-V168M cRNA. This indicated that AQP2-V168M is impaired in its transport to the membrane compared with wt-AQP2, but that its trafficking to the plasma membrane is significantly better than of AQP2-T126M. Determining the conferred water permeabilities (Figure 2) for similar plasma membrane expression levels of wt-AQP2 and AQP2-V168M revealed that the ability of AQP2-V168M to confer water permeation is about 58% of that of wt-AQP2.

Immunocytochemical analysis of these oocytes showed a dispersed intracellular expression for AQP2-V168M and AQP2-T126M, although AQP2-V168M also revealed a weak plasma membrane expression (Figure 4, A and B), which is
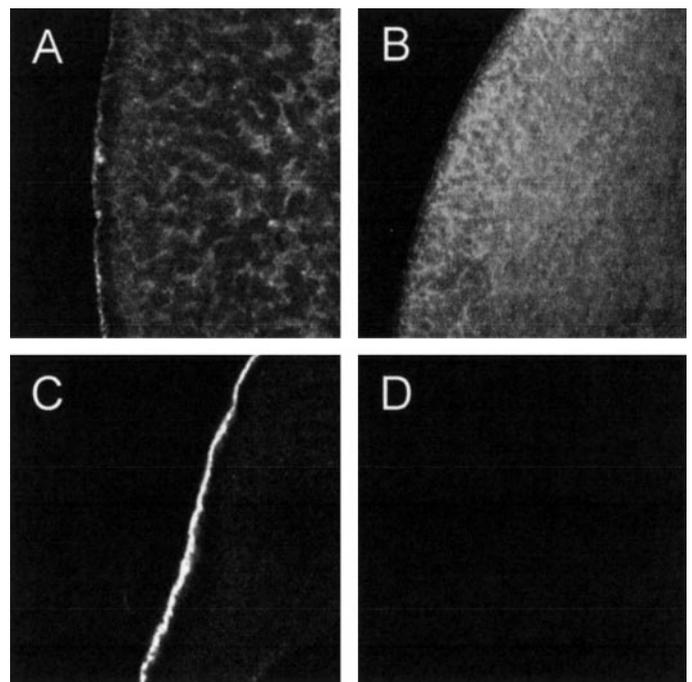
Figure 4. Localization of aquaporin-2 (AQP2)–V168M in oocytes. Two days after injection, uninjected oocytes (D) and oocytes injected with 1 ng cRNA encoding AQP2-V168M (A), wt-AQP2 (C), or 3 ng for AQP2-T126M (B) were fixed in paraformaldehyde and embedded in paraffin; 5-μm sections were cut and incubated with rabbit α-AQP2 antibodies, followed by Alexa-594.

consistent with ER retention for both mutants and a partial plasma membrane expression of AQP2-V168M. As anticipated, wt-AQP2 was only detected in the plasma membrane (Figure 4C). Uninjected oocytes, which were taken as controls, did not show any staining (Figure 4D), confirming the specificity of the AQP2 antibodies.

Effect of the Mutation on Aquaporin-2 Function on Water Excretion and dDAVP Response

Family members who were homozygous for the mutation (III-3, III-5, and III-7) had a mean 24-h urinary volume of 10,542 ± 941 ml and mean Uosm of 69 ± 9 mOsm/kg (Table 1). The mean 24-h urine volume of the family members heterozygous for the mutation (II-2 and III-9) was 1500 ± 0 ml, which was similar to spousal controls (III-4 and III-6) 1275 ± 530 ml ($P = 0.66$). The mean Uosm in the heterozygotes was 648 ± 41 mOsm/kg, also similar to the controls (641 ± 257 mOsm/kg; $P = 0.96$).

Two homozygous (III-5 and III-7) and one heterozygous (II-2) family members participated in the water deprivation study (Table 2 and Figure 5). All of the baseline electrolytes, blood urea nitrogen, glucose, creatinine, and calcium levels were within normal limits. After the Sosm reached 300 mOsm/kg, and 2 h after 1 μg of dDAVP was provided intravenously, the homozygotes had Uosm of 146 mOsm/kg and 216 mOsm/kg. The urine volume did not decrease after the dose of dDAVP. In III-7, the mean urine volume before dDAVP was 417 ± 40 ml/h, and the mean post-dDAVP treatment was 720 ± 113 ml/h ($P = 0.14$). In III-5, the urine volumes were 625 ± 240 ml/h and 700 ± 283 ml/h ($P = 0.78$) before and after dDAVP, respectively. The 2-h Uosm in the heterozygote subject was 521 mOsm/kg, consistent with partial NDI. Only one of the homozygous individuals (III-5) had high levels of AVP (29.6 pg/ml) at the maximum Sosm of 320 mOsm/kg.

Three homozygous (III-3, III-5, and III-7), two heterozygous (II-2 and III-9), and two control (III-4 and III-6) family members participated in the dDAVP challenge test (Table 3 and Figure 6). Baseline electrolytes, blood urea nitrogen, glucose, creatinine, and calcium levels were within normal limits. The father (II-2) had elevated systolic and diastolic BP, with means of 152 ± 12 mm Hg and 79 ± 6 mm Hg, respectively. In the homozygous individuals, the mean Uosm was 70 ± 3 mOsm/kg at baseline, 116 ± 16 mOsm/kg after the first dose, and 173 ± 15 mOsm/kg after the second dose of dDAVP. In these same individuals, the water intake had decreased, with a mean intake of 252 ± 131 ml/h between the first and second dose of dDAVP and 199 ± 143 ml/h between the second and

third dose of dDAVP ($P = 0.07$). The mean urine volume did not decrease significantly between the first and second dose of dDAVP ($P = 0.81$).

The heterozygous individuals had a baseline urinary osmolality of 762 ± 274 mOsm/kg, which was not significantly different from 697 ± 34 mOsm/kg observed in controls ($P = 0.76$). Similarly, the results of the urinary osmolality after the first dose of dDAVP between the heterozygotes (874 ± 247 mOsm/kg) and controls (803 ± 46 mOsm/kg) were not significantly different ($P = 0.78$). One of the controls and one of the heterozygous required only one dose of dDAVP of 1 μg to increase urine osmolarity above 800 mOsm/kg.

Genetic Studies of the Mexican Town

Buccal cell samples were collected from 222 individuals from the town of origin of the family. We evaluated DNA from 218 of the samples. We were unable to evaluate four samples (2%) because of poor DNA harvest. Fifty-nine percent of the samples were from women, and 41% were from men. The mean age of the individuals sampled was 48 ± 20 yr (women, 48 ± 19 yr; men, 49 ± 20 yr). The age distribution of the sample had a preponderance of older individuals when compared with the Mexican estate where the town is located, the Nuevo Leon estate (Figure 7). Of the total 218 samples processed, 151 samples (69%) did not have the V168M mutation in AQP2. Sixty-five samples (30%) were heterozygous for the V168M *AQP2* mutation, and two samples (1%) were homozygous for the mutation. The C/T polymorphism immediately adjacent to the mutation was also observed in this population at a frequency of 8% T allele. The polymorphism did not associate with the mutation and was in Hardy-Weinberg equilibrium.

The gender distribution of the group heterozygous for the mutation was similar to the sample: 63% women and 37% men. The mean age of the individuals heterozygous for the V168M was 59 yr ± 20, 11 ± 0 yr older than the sample ($P < 0.01$). The two homozygous individuals were both women, 25 and 60 yr of age. Deviation from Hardy-Weinberg equilibrium for the V168M polymorphism was evaluated, and the population was found not to deviate significantly from the expectation on the basis of the marginal genotype frequencies ($\chi^2 = 3.094$, $P = 0.080$).

Discussion

We have characterized a V168M mutation in AQP2 in a family with recessive NDI from Mexico. This mutation caused a partial defect in the affected individuals. No previous *AQP2*

Table 1. Results of 24-h urine collection

Individuals	M/M			V/M		V/V	
	III-7	III-5	III-3	III-9	II-2	III-6	III-4
Urine volume (ml/24 h)	11,600	9800	10,225	1500	1500	1650	900
Hourly volume (ml)	483	408	426	63	63	69	38
Uosm (mOsm/kg)	62	66	80	583	712	459	822

Table 2. Results of water deprivation test[a]

| | Saline Infusion | | | | After 1 μg dDAVP | |
	Baseline	1h	2h	3h	1h	2h
III 7 (M/M)						
UOsm (mOsm/kg)	65	85	98	0	130	146
Sosm (mOsm/kg)	304	304	307	0	310	317
UVol (ml/h)	410	380	460		640	800
AVP (pg/ml)	4.2		3.9			
III5 (M/M)						
UOsm (mOsm/kg)	76	110	142	185	224	216
Sosm (mOsm/kg)	291	294	309	321	323	316
UVol (ml/h)	400	450	750	900	900	500
AVP (pg/ml)	2.9		29.6			
II/2 (V/M)						
UOsm (mOsm/kg)	404	412	493	508	502	521
Sosm (mOsm/kg)	289	297	298	307	311	309
Uvol (ml/h)	120	110	120	110	220	180
AVP (pg/ml)	<1.0		2.8			

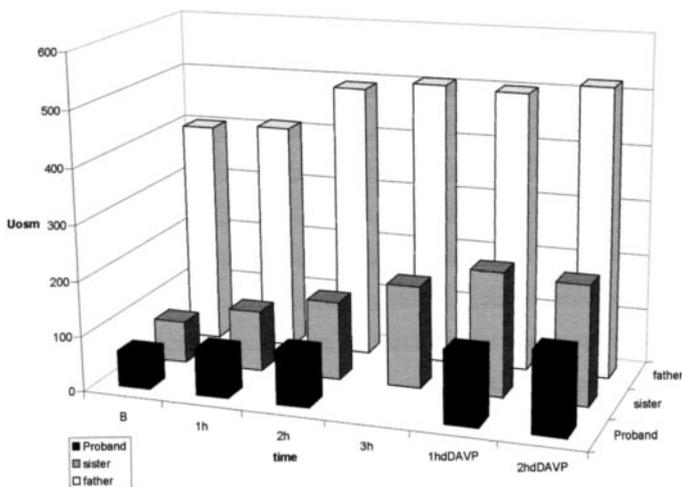[a] Uosm, urine osmolality; Sosm, serum osmolality; Uvol, urine volume; AVP, vasopressin.

Figure 5. Results of the water deprivation test in three family members.

mutation has been described in individuals of Mexican or Latin American ethnic origin, although five Brazilian families of European ancestors were reported with *AQP2* mutations (15). The V168M mutation in AQP2 has been described in a family of European descent, in which clinically affected individuals were compound heterozygotes for the V168M and a S126P mutation (16). The functional effect of the V168M mutation on AQP2 was not previously studied, as opposed to the S216P mutation (3,4). The studies in oocytes and the immunocytochemistry results revealed that AQP2-V168M was retained in the ER, a molecular defect that is common with AQP2 mutants causing recessive NDI (30). However, compared with AQP2-T126M, which is another mutant in recessive NDI, AQP2-V168M is clearly less impaired in its trafficking to the plasma

membrane, because even at lower total membrane expression levels, more AQP2-V168M is detected in the plasma membrane (Figures 3 and 4). Because it is well known that an impaired exit from the ER is a consequence of misfolding and retention by molecular chaperones that exert the ER quality control, this reduced level of ER retention of AQP2-V168M compared with AQP2-T126M might indicate that AQP2-168M is less misfolded than AQP2-T126M. This is underscored by the single channel permeability of about 58% for AQP2-V168M in relation to wt-AQP2, whereas 20% was found for AQP2-T126M (36). Our oocyte studies do not reveal whether AQP2-V168M mRNA and protein are less stable than of AQP2-T126M. However, if these stabilities are similar *in vivo*, the observed differences in permeability and trafficking to the plasma membrane of these AQP2 mutants would provide a cell biologic explanation for the partial improvement of the urinary concentrating capacity and thirst reduction with the use of dDAVP in the AQP2-V168M individuals and the absence of any increased urine-concentrating ability with dDAVP in the NDI patients encoding AQP2-T126M (14).

Only homozygous individuals in the family were clinically affected with partial NDI, a finding based on very high 24-h urine volumes and low urinary osmolalities in the NDI range, even after water deprivation and administration of dDAVP. The baseline AVP levels were lower than expected for patients with severe NDI. In addition, during the water deprivation and saline infusion, the AVP levels only increased outside the normal range for the Sosm in III-5, in whom the second AVP drawn was 29.6 pg/ml for a Sosm of 320 mOsm/kg, supporting the possibility of a milder type of disease.

Family members heterozygous for the *AQP2* mutation (II-2 and III-9) were not clinically affected and had Uosm and urine volumes similar to those of controls. The father (II-2), 55 yr

Table 3. dDAVP challenge test[a]

	M/M			V/M		V/V	
	III-7	III-5	III-3	III-9	II-2	III-6	III-4
Uosm base (mOsm/kg)	69	73	67	956	568	721	673
Sosm base (mOsm/kg)	294	293	289	296	294	296	285
First dDAVP dose (mcg)	5	5	5	1	1	1	1
fluid intake (ml/h)	240	128	388	145	261	118	307
urine volume (cc)	1800	600	1552	125	516	200	452
urine output (ml/h)	450	150	388	31	129	50	113
Uosm (mOsm/kg)	109	135	105	1049	699	770	835
Second dDAVP dose (μg)	36.3	19.4	24	None	4	4	None
fluid intake (ml/h)	160	80	358		110	163	
urine volume (cc)	500	725	1434		225	180	
urine output (ml/h)	167	242	478		75	60	
Uosm (mOsm/kg)	179	183	156		692	956	
Sosm (mOsm/kg)	301	303	297		291	296	

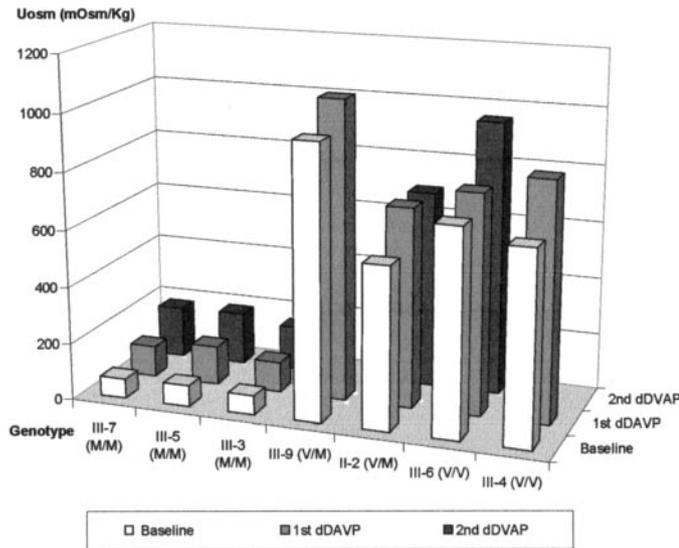[a] Uosm, urine osmolality; Sosm, serum osmolality.

Figure 6. Results of the dDAVP (1-desamino-8-D-arginine-vasopressin) challenge test in affected and unaffected family members.
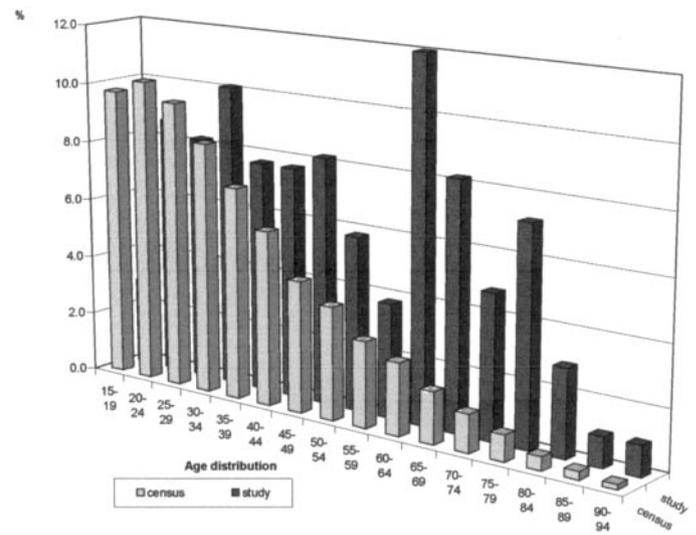

Figure 7. Age distribution of the sample collected in the Mexican town compared with the XII Censo General de Poblacion y Vivienda (Nuevo Leon) 2000 Census.

old, showed partial NDI in two separate tests with a maximal urine osmolarity of 699 mOsm/Kg, when a normal 80-yr-old individual should be able to reach at least a urinary osmolality of 823 mOsm/Kg (22,24,29). It is possible that he had limited urinary concentrating capacity as a result of other causes, such as long-standing hypertension.

Usually NDI does not respond to treatment with dDAVP unless some vasopressin and AQP2 activity remains at the kidney level (39). Because of the rarity of this mutation, we had no families with other mutations available to be used as controls. The majority of the subjects with *AQP2* mutations did not respond to dDAVP (3,4,11–13,15,16). Three previous studies in patients with *AQP2* mutations have demonstrated partial diabetes insipidus, one individual with an autosomal recessive

mutation (9) and the other two individuals with autosomal dominant mutations (17,20). *AVPR2* mutations have also been reported to produce partial response to dDAVP with milder phenotypes and with mothers affected with partial NDI (15,16).

The homozygotes in our study repeatedly reported subjective improvement of thirst and urination when dDAVP was used at home, as expected if principal collecting duct cells are still responding demonstrating some AQP2 function. This was confirmed by the oocyte functional studies and the clinical studies. The Uosm increased marginally with water deprivation and dDAVP, and some functional aquaporin-2 was found in the plasma membrane of the oocytes. The urinary output in homozygotes treated with dDAVP or water deprivation was

not significantly changed, but a decrease in their water intake was observed during the dDAVP challenge test.

None of the affected individuals in the Mexican family had mental or developmental abnormalities despite symptoms of polyuria and polydipsia from an early age. In addition, two undiagnosed affected individuals were identified in the Mexican town. These results indicate that some *AQP2* mutations causing partial recessive NDI permits normal growth and mental development, even if the disease is undiagnosed. Members of other large kindreds affected with NDI have reported mental retardation or failure to thrive as a result of untreated NDI. In a family with recessive *AQP2* mutations, 6 of 11 individuals had slow psychomotor development or mental retardation and short stature (2). Another study reported the death of three children with symptoms of diabetes insipidus in a family with recessive *AQP2* gene mutations (4). None of the affected individuals in the Mexican family had mental or developmental abnormalities, despite symptoms of polyuria and polydipsia from an early age. In addition, two undiagnosed affected individuals were identified in the Mexican town.

The fact that each of the parents of the proband carried the same rare recessive *AQP2* mutation led us to do a genetic epidemiologic study in the town of origin. The population sampled in the town was older when compared with the national census. In addition, more women than men were sampled. Young men in the town were often employed as seasonal migrant farm workers, and this fact may have affected the population sampled. Thirty percent of the individuals in the sample were heterozygous for the mutation, and 1% were homozygous. The frequency of individuals affected by mutations in the *AQP2* gene in the general population of Mexico is unknown, but it is likely to be less than one in a million (8,40). The high frequency of the mutant allele in the population (15.8%) suggests a founder effect of the mutation in the population. The extraordinarily high prevalence of this recessive mutation in the town, along with the distribution of the mutation along all ages, suggests a relatively high risk of having more affected individuals born in this town. The heterozygote individuals had a similar gender distribution when compared with the entire sample, but had a mean age 11 yr older, which could be suggestive of increased admixture with other populations in the younger generations.

The polymorphism immediately adjacent to the mutation was clearly in equilibrium and was on a separate allele from the mutation. None of the individuals in the family had the polymorphism at nucleotide 501 present in their DNA. In addition, none of the individuals in the town that were homozygous for the polymorphism had the mutation. The health care workers in the town were previously unaware of the presence of NDI in the community. The life-threatening complications of NDI can be easily treated if an individual and the health care providers are aware of the condition. As an attempt to increase the alertness in the town about the disease, we have provided medical information concerning this condition to the health care workers and the population.

Conclusion

In summary, individuals affected with partial NDI in a family of Mexican origin were found to be homozygous for a V168M mutation in AQP2. Consistent with this phenotype, AQP2-V168M was retained in the ER of oocytes, but was still partially functional and less impaired in its trafficking to the cell surface than another functional AQP2 mutant involved in severe recessive NDI. The affected homozygous individuals reported subjective improvement of thirst and urination with the use of dDAVP. This finding was consistent with the clinical studies, which showed partial NDI. dDAVP treatment partially increased Uosm and decreased fluid intake. Heterozygotes compared with controls showed no urinary concentrating defects. In the genetic epidemiologic study of the town of origin, we found a high prevalence of the V168M mutation, suggesting a founder effect. The mutation in the population was in Hardy-Weinberg equilibrium. Affected individuals had no neurologic deficits from untreated NDI. This raises the speculation that undiagnosed cases of NDI may exist in other populations. Preventive and educational measures were implemented in the town with the cooperation of the local health authorities.

References

1. Robertson GL: Diabetes insipidus. *Endocrinol Metab Clin North Am* 24: 549–572, 1995
2. Hochberg Z, Van Lieburg A, Even L, Brenner B, Lanir N, van Oost BA, Knoers NV: Autosomal recessive nephrogenic diabetes insipidus caused by an aquaporin-2 mutation. *J Clin Endocrinol Metab* 82: 686–689, 1997
3. Pasel K, Schulz A, Timmermann K, Linnemann K, Hoeltzenbein M, Jaaskelainen J, Gruters A, Filler G, Schoneberg T: Functional characterization of the molecular defects causing nephrogenic diabetes insipidus in eight families. *J Clin Endocrinol Metab* 85: 1703–1710, 2000
4. van Lieburg AF, Verdijk MA, Knoers VV, van Essen AJ, Proesmans W, Mallmann R, Monnens LA, van Oost BA, van Os CH, Deen PM: Patients with autosomal nephrogenic diabetes insipidus homozygous for mutations in the aquaporin 2 water-channel gene. *Am J Hum Genet* 55: 648–652, 1994
5. Crawford JD, Bode HH: Diabetes and the amplifier hypothesis. *N Engl J Med* 282: 1266–1267, 1970
6. Niaudet P, Dechaux M, Trivin C, Loirat C, Broyer M: Nephrogenic diabetes insipidus: Clinical and pathophysiological aspects. *Adv Nephrol Necker Hosp* 13: 247–260, 1984
7. Morello JP, Bichet DG: Nephrogenic diabetes insipidus. *Annu Rev Physiol* 63: 607–630, 2001
8. van Os CH, Deen PM: Aquaporin-2 water channel mutations causing nephrogenic diabetes insipidus. *Proc Assoc Am Physicians* 110: 395–400, 1998
9. Canfield MC, Tamarappoo BK, Moses AM, Verkman AS, Holtzman EJ: Identification and characterization of aquaporin-2 water channel mutations causing nephrogenic diabetes insipidus with partial vasopressin response. *Hum Mol Genet* 6: 1865–1871, 1997
10. Deen PM, Verdijk MA, Knoers NV, Wieringa B, Monnens LA, van Os CH, van Oost BA: Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* 264: 92–95, 1994

11. Goji K, Kuwahara M, Gu Y, Matsuo M, Marumo F, Sasaki S: Novel mutations in aquaporin-2 gene in female siblings with nephrogenic diabetes insipidus: Evidence of disrupted water channel function. *J Clin Endocrinol Metab* 83: 3205–3209, 1998

12. Hochberg Z, Even L, Danon A: Amelioration of polyuria in nephrogenic diabetes insipidus due to aquaporin-2 deficiency. *Clin Endocrinol Oxf* 49: 39–44, 1998

13. Lin SH, Bichet DG, Sasaki S, Kuwahara M, Arthus MF, Lonergan M, Lin YF: Two novel aquaporin-2 mutations responsible for congenital nephrogenic diabetes insipidus in Chinese families. *J Clin Endocrinol Metab* 87: 2694–2700, 2002

14. Mulders SM, Knoers NV, van Lieburg AF, Monnens LA, Leumann E, Wuhl E, Schober E, Rijss JP, van Os CH, Deen PM: New mutations in the AQP2 gene in nephrogenic diabetes insipidus resulting in functional but misrouted water channels. *J Am Soc Nephrol* 8: 242–248, 1997

15. Rocha JL, Friedman E, Boson W, Moreira A, Figueiredo B, Liberman B, de Lacerda L, Sandrini R, Graf H, Martins S, Punales MK, De Marco L: Molecular analyses of the vasopressin type 2 receptor and aquaporin-2 genes in Brazilian kindreds with nephrogenic diabetes insipidus. *Hum Mutat* 14: 233–239, 1999

16. Vargas-Poussou R, Forestier L, Dautzenberg MD, Niaudet P, Dechaux M, Antignac C: Mutations in the vasopressin V2 receptor and aquaporin-2 genes in 12 families with congenital nephrogenic diabetes insipidus. *J Am Soc Nephrol* 8: 1855–1862, 1997

17. Mulders SM, Bichet DG, Rijss JP, Kamsteeg EJ, Arthus MF, Lonergan M, Fujiwara M, Morgan K, Leijendekker R, van der SP, van Os CH, Deen PM: An aquaporin-2 water channel mutant which causes autosomal dominant nephrogenic diabetes insipidus is retained in the Golgi complex. *J Clin Invest* 102: 57–66, 1998

18. Kamsteeg EJ, Wormhoudt TA, Rijss JP, van Os CH, Deen PM: An impaired routing of wild-type aquaporin-2 after tetramerization with an aquaporin-2 mutant explains dominant nephrogenic diabetes insipidus. *EMBO J* 18: 2394–2400, 1999

19. Marr N, Bichet DG, Lonergan M, Arthus MF, Jeck N, Seyberth HW, Rosenthal W, van Os CH, Oksche A, Deen PM: Heteroligomerization of an aquaporin-2 mutant with wild-type aquaporin-2 and their misrouting to late endosomes/lysosomes explains dominant nephrogenic diabetes insipidus. *Hum Mol Genet* 11: 779–789, 2002

20. Kuwahara M, Iwai K, Ooeda T, Igarashi T, Ogawa E, Katsushima Y, Shinbo I, Uchida S, Terada Y, Arthus MF, Lonergan M, Fujiwara TM, Bichet DG, Marumo F, Sasaki S: Three families with autosomal dominant nephrogenic diabetes insipidus caused by aquaporin-2 mutations in the C-terminus. *Am J Hum Genet* 69: 738–748, 2001

21. Kamsteeg EJ, Bichet DG, Konings IB, Nivet H, Lonergan M, Arthus MF, van Os CH, Deen PM: Reversed polarized delivery of an aquaporin-2 mutant causes dominant nephrogenic diabetes insipidus. *J Cell Biol* 163: 1099–1109, 2003

22. Zerbe RL, Robertson GL: A comparison of plasma vasopressin measurements with a standard indirect test in the differential diagnosis of polyuria. *N Engl J Med* 305: 1539–1546, 1981

23. Baylis PH, Robertson GL: Plasma vasopressin response to hypertonic saline infusion to assess posterior pituitary function. *J R Soc Med* 73: 255–260, 1980

24. Tryding N, Berg B, Ekman S, Nilsson JE, Sterner G, Harris A: DDAVP test for renal concentration capacity: Age-related reference intervals. *Scand J Urol Nephrol* 22: 141–145, 1988

25. Tryding N, Sterner G, Berg B, Harris A, Lundin S: Subcutaneous and intranasal administration of 1-deamino-8-D-arginine vasopressin in the assessment of renal concentration capacity. *Nephron* 45: 27–30, 1987

26. Monson JP, Richards P: Desmopressin urine concentration test. *Br Med J* 1: 24, 1978

27. Somerfield SD, Hocken AG: Desamino arginine vasopressin (DDAVP) as a diagnostic agent. *N Z Med J* 86: 472–473, 1977

28. Fjellestad-Paulsen A, Hoglund P, Lundin S, Paulsen O: Pharmacokinetics of 1-deamino-8-D-arginine vasopressin after various routes of administration in healthy volunteers. *Clin Endocrinol (Oxf)* 38: 177–182, 1993

29. Jassal SV, Oreopoulos DG: The aging kidney. *Geriatr Nephrol Urol* 8: 141–147, 1998

30. Marr N, Bichet DG, Hoefs S, Savelkoul PJ, Konings IB, De Mattia F, Graat MP, Arthus MF, Lonergan M, Fujiwara TM, Knoers NV, Landau D, Balfe WJ, Oksche A, Rosenthal W, Muller D, van Os CH, Deen PM: Cell-biologic and functional analyses of five new aquaporin-2 missense mutations that cause recessive nephrogenic diabetes insipidus. *J Am Soc Nephrol* 13: 2267–2277, 2002

31. Lum A, Le Marchand L: A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. *Cancer Epidemiol Biomarkers Prev* 7: 719–724, 1998

32. Garcia-Closas M, Egan KM, Abruzzo J, Newcomb PA, Titus-Ernstoff L, Franklin T, Bender PK, Beck JC, Le Marchand L, Lum A, Alavanja M, Hayes RB, Rutter J, Buetow K, Brinton LA, Rothman N: Collection of genomic DNA from adults in epidemiological studies by buccal cytobrush and mouthwash. *Cancer Epidemiol Biomarkers Prev* 10: 687–696, 2001

33. Snedecor GW, Cochran WG: *Statistical Methods*, 8th Ed., Ames, Iowa State University Press, 1989

34. Warnes G, Leisch F. *The Genetics Package for R-Plus* (1.6-40). www.R-project.org, GNU General Public License V2, 3-19-2003

35. Instituto Nacional de Estadistica GeIM: Estados Unidos Mexicanos: *XII censo general de poblacion y vivienda*, Mexico City, Mexico, Aguascalientes, Ags., Instituto Nacional de Estadistica, Geografia e Informatica, 2000

36. Marr N, Kamsteeg EJ, van Raak M, van Os CH, Deen PM: Functionality of aquaporin-2 missense mutants in recessive nephrogenic diabetes insipidus. *Pflugers Arch* 442: 73–77, 2001

37. Tamarappoo BK, Yang B, Verkman AS: Misfolding of mutant aquaporin-2 water channels in nephrogenic diabetes insipidus. *J Biol Chem* 274: 34825–34831, 1999

38. Kamsteeg EJ, Deen PM: Importance of aquaporin-2 expression levels in genotype-phenotype studies in nephrogenic diabetes insipidus. *Am J Physiol Renal Physiol* 279: F778–F784, 2000

39. Sadeghi H, Robertson GL, Bichet DG, Innamorati G, Birnbaumer M: Biochemical basis of partial nephrogenic diabetes insipidus phenotypes. *Mol Endocrinol* 11: 1806–1813, 1997

40. Arthus MF, Lonergan M, Crumley MJ, Naumova AK, Morin D, De Marco LA, Kaplan BS, Robertson GL, Sasaki S, Morgan K, Bichet DG, Fujiwara TM: Report of 33 novel AVPR2 mutations and analysis of 117 families with X-linked nephrogenic diabetes insipidus. *J Am Soc Nephrol* 11: 1044–1054, 2000