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Autosomal Recessive Nephrogenic Diabetes Insipidus Caused by an Aquaporin-2 Mutation*

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ABSTRACT

Vasopressin V_2 receptors, expressed from an x-chromosomal gene, are involved in antidiuresis, but also in release of coagulation factor VIII and von Willebrand factor (vWF). The present study describes autosomal recessive nephrogenic diabetes insipidus (NDI) in a large cluster of patients in Israel's Lower-Galilee. Evidence for an intact V_2 receptor was concluded by their normal increase in factor VIII and vWF after desmopressin infusion. Thus, in these patients a defect in the pathway beyond the V_2 receptor was suspected. The recent cloning of the human Aquaporin-2 gene enabled us to test this gene as a candidate for such a postreceptor defect. Direct sequencing of the Aquaporin-2 gene revealed a G298T substitution causing a Gly100Stop nonsense mutation in the third transmembrane region. Because this putative disease-causing mutation was identified in index patients of different families, we suggest that all patients are descendants of a common ancestor. Thus, this new entity is characterized by an autosomal recessive NDI. The differential response of clotting factors and urine osmolality to desmopressin may provide a simple tool for clinical diagnosis of a V₂-postreceptor defect. The early stop-codon of Aquaporin-2 results in complete resistance to vasopressin antidiuretic effect. (J Clin Endocrinol Metab 82: 686-689, 1997)

ASOPRESSIN exerts its effects through stimulation of at least two types of receptors: V_1 receptors mediate the pressor response of this hormone and other actions, such as glycogenolysis and platelet aggregation. V₂ receptors are involved in antidiuresis, but also in release of coagulation factor VIII, von Willebrand factor (vWF), and tissue-type plasminogen activator (t-PA) (1). Mutations in the x-chromosomal V₂ receptor result in X-linked nephrogenic diabetes insipidus (NDI) (2, 3). Biochemically, the V₂ receptor defect of X-linked NDI is reflected by a blunted antidiuretic response to the V_2 receptor agonist desmopressin. Moreover, patients with X-linked NDI do not show an increase of coagulation and fibrinolytic factors to desmopressin administration (4). The X-linked type of NDI is the most frequent form of the disease, but some families have been described in which NDI shows an autosomal recessive mode of inheritance. In previous studies, we have found evidence for an intact V₂ receptor in four NDI patients, as concluded by their normal increase in factor VIII and vWF after desmopressin infusion (5, 6). Thus, in these patients a defect in the pathway beyond the V_2 receptor was suspected. By screening kidney cDNA and cosmid libraries with a rat Aquaporin-2 cDNA as a probe, the human Aquaporin-2 (AQP2) gene was identified, assigned to chromosome 12, and found to be mutated in a patient with autosomal recessive NDI (7). Subsequently, three additional NDI families with mutations in the AQP2 gene have been reported (8).

The present study describes autosomal recessive NDI in a large cluster of patients in Israel's Lower-Galilee. We have identified a new mutation in the AQP2 gene. Because this putative disease-causing mutation was identified in index patients of different families, we suggest that all patients are descendants of a common ancestor.

Subjects and Methods

The pedigrees of the patients are depicted in Fig. 1. The families are of Bedouin-Arab origin, where first cousin marriage is traditional. Although we have not been able to trace the relationship of these families with each other, such relation can be assumed to exist as members of these three families live within an area of 20 km.

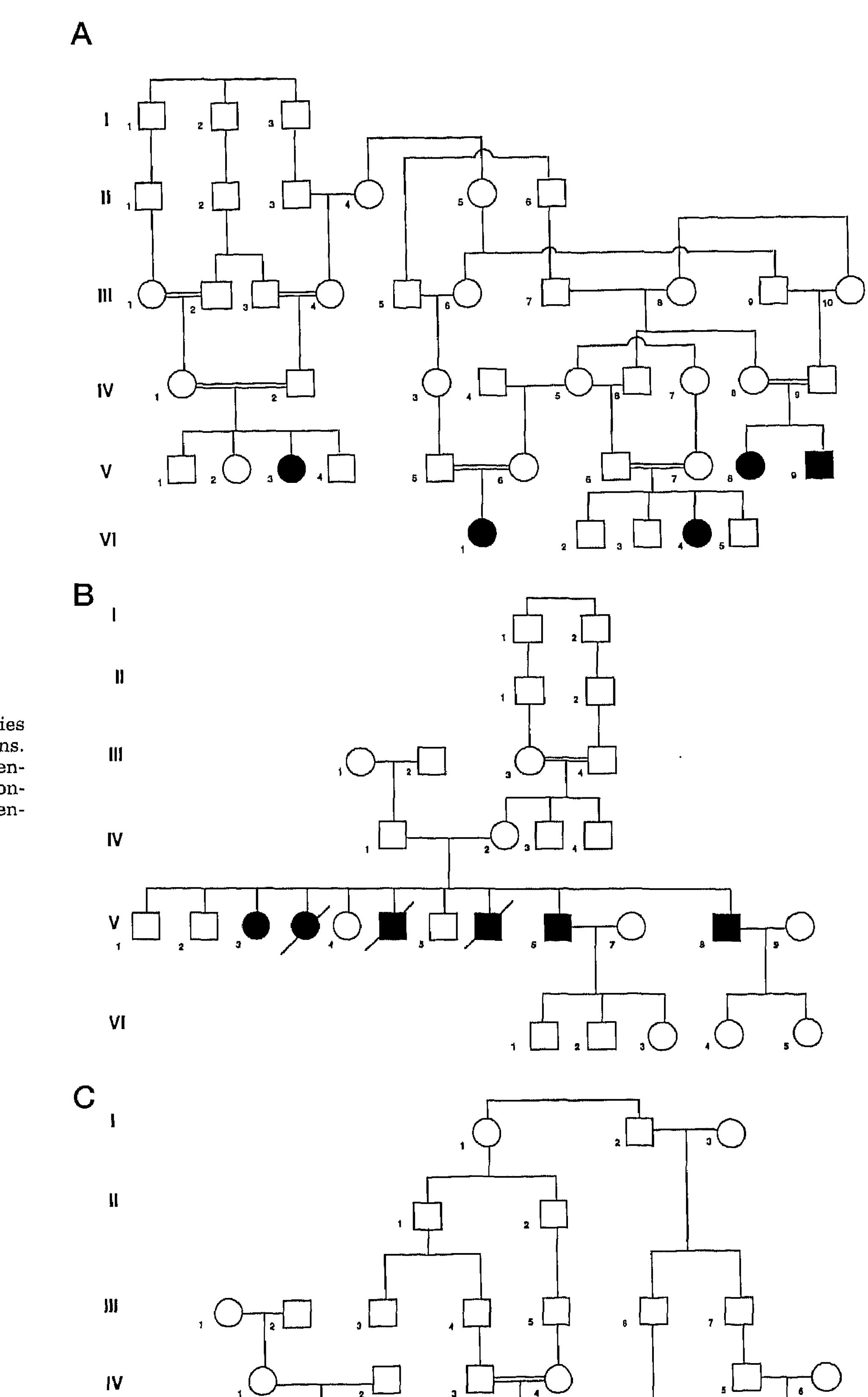
Presenting with neonatal fever and vomiting (6/11 patients) or failure to thrive (4/11 patients), NDI was diagnosed by demonstration of polyuria of 161–250 mL/kg/day, low urinary osmolality, with maximal concentration to 52–102 mOsm/kg, high simultaneous plasma osmolality of 296–326 mOsm/kg, and serum sodium concentration of 147–168 mEq/L and lack of response to vasopressin. The clinical picture was also characterized by fetal distress (3/5 patients), short stature with a mean height of -1.05 sp, slow psychomotor development and mental retardation (6/11 patients), and psychosis in one patient, who presented with extreme and aggressive anger at night, which might have been related to thirst.

Two pairs of the obligatory heterozygous parents of affected patients were screened for a possible concentration defect. Their fasting serum sodium (139–142 mEq/L) urine osmolality (780–940 mOsm/kg) was normal, as were the 24-h urine volumes (21–29 mL/kg/d).

Desmopressin infusion test

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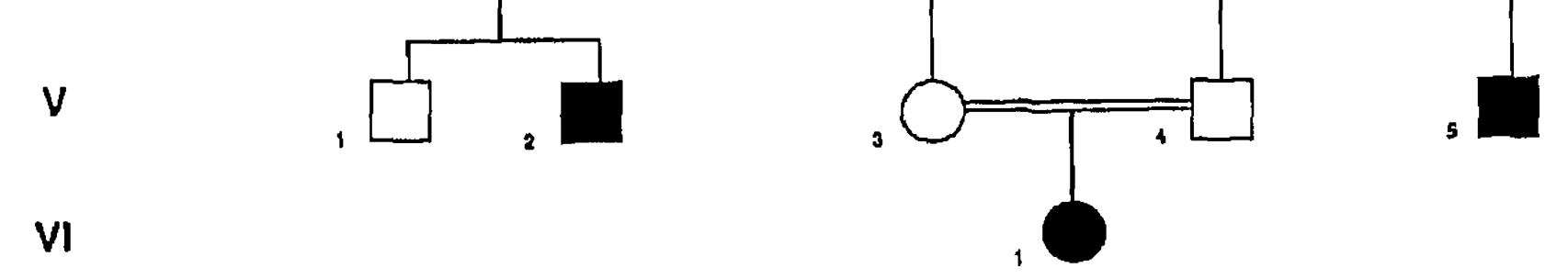
Received May 21, 1996. Revision received August 5, 1996. Re-revision received October 10, 1996. Accepted November 7, 1996. Address correspondence and requests for reprints to: Dr. Z. Hochberg, Department of Pediatrics, POB 9649, Haifa 31096, Israel. Presented at the annual meeting of The European Society of Pediatric Endocrinology, Montpellier, France, September 1996. Desmopressin infusion tests were performed in 7 of the patients and 5 healthy controls. Before desmopressin infusion a water-load of 600 mL/m² was given orally to suppress endogenous vasopressin. Basal urine was collected for an hour. Starting at zero time, desmopressin (Minirin, Ferring, Malmo, Sweden) was infused over 10 min in a dose of 0.3 μ g/kg. Urine and blood were collected at 0, 60, and 120 min. Plasma and urine osmolality were measured with a cryosmometer.



AQUAPORIN-2

FIG. 1. Pedigrees of the three families with NDI and Aquaporin-2 mutations. Patients designated in the text are identified by the family initial-generationpatients number in the respective generation (i.e. K-VI-2).

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Coagulation factors

Factor VIII activity was measured by a single-stage assay, as previously reported (9). The normal range in 30 healthy controls was 50–150 U/dL. vWF antigen was measured by an enzyme-immunoassay (10), with reagents purchased from Stago (Asnieres, France).

Mutation analysis

Chromosomal DNA was isolated from blood using the salt extraction method (11). Amplification of 100 ng of genomic DNA was performed in 100 μ L, using primers flanking exon 1 of the AQP2 gene. After purification of the polymerase chain reaction (PCR) product, the same primers were used to sequence both strands in the cycle sequencing reaction with the fluorescence-based Applied Biosystems model 373A DNA-sequencing system (7). Restriction mapping of the crude exon I PCR product was carried out with *MspI*, and the digestion products were resolved on 2% agarose and visualized with ethidiumbromide. osmolality was 288 \pm 2 mOsm/kg in controls and 293 \pm 7 mOsm/kg in NDI patients (P < 0.05). Urinary osmolality increased from 285 \pm 112 to 640 \pm 92 mOsm/kg in controls, but remained dilute at 81 \pm 9 mOsm/kg in NDI patients. Clotting factor VIII increased in control from 75 \pm 5 to 204 \pm 73 U/dl and in NDI patients from 132 \pm 23 (P < 0.01) to 293 \pm 175 U/dl (NS). vWF-antigen increased in control from 178 \pm 42 (P < 0.02) to 302 \pm 130 U/dL (P < 0.05).

Direct DNA sequencing of the AQP2 gene of the index patients VI-1 of the H family and VI-4 of the G family revealed a G298T substitution causing a Gly100Stop nonsense mutation in the third transmembrane region. As this mutation destroys the *MspI* site present in the first exon, a simple restriction site PCR test for carriership was possible. In both patients the 474 bp PCR fragment for exon I could not be cut with *MspI*, confirming the homozygous nature of the mutation found. In the parents of both patients the 474 bp fragment and a 370 bp fragment were visible on EtBr-stained

Results

In Fig. 2, the results of the desmopressin infusion test in seven patients and five controls are presented. Basal plasma

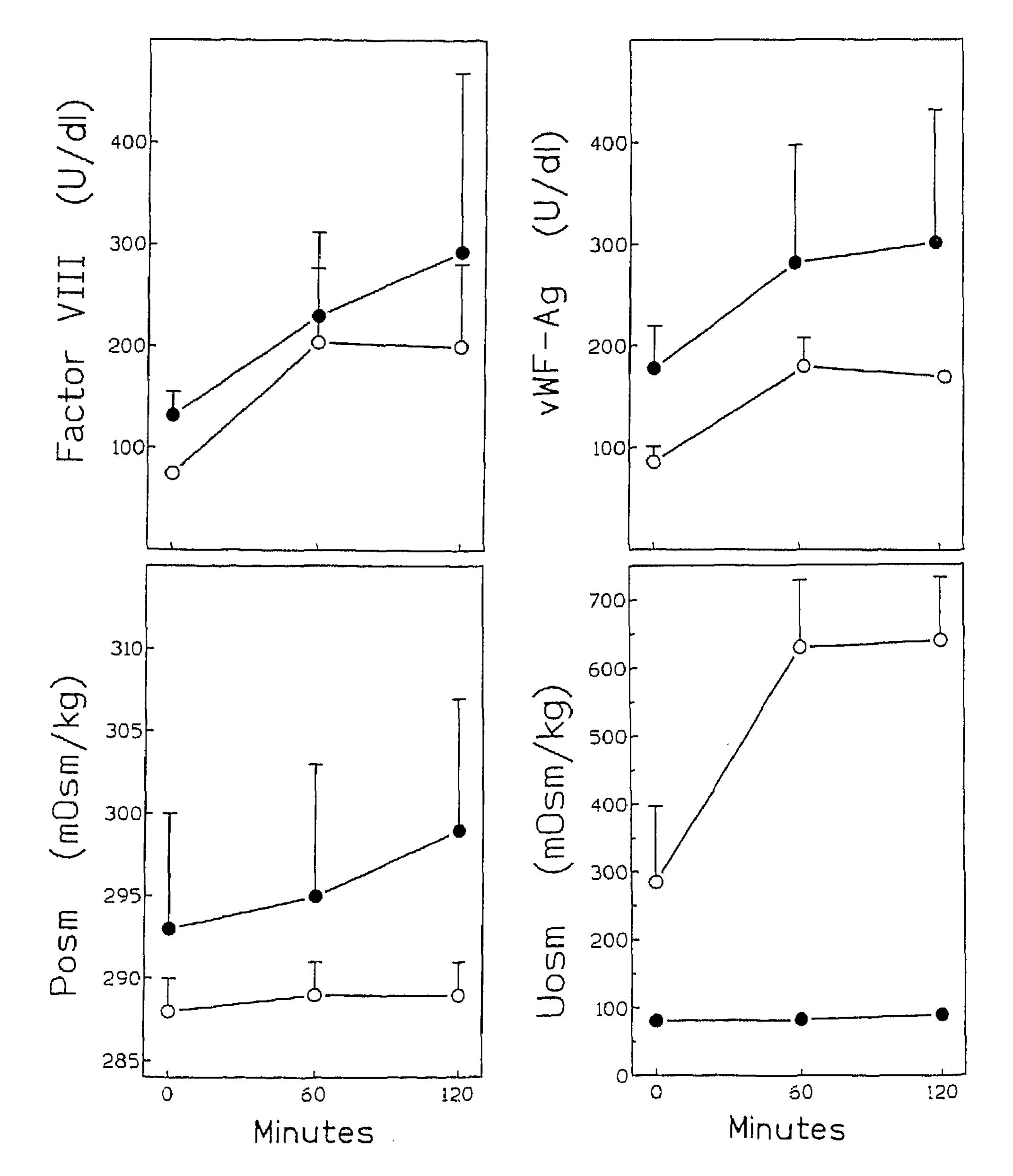
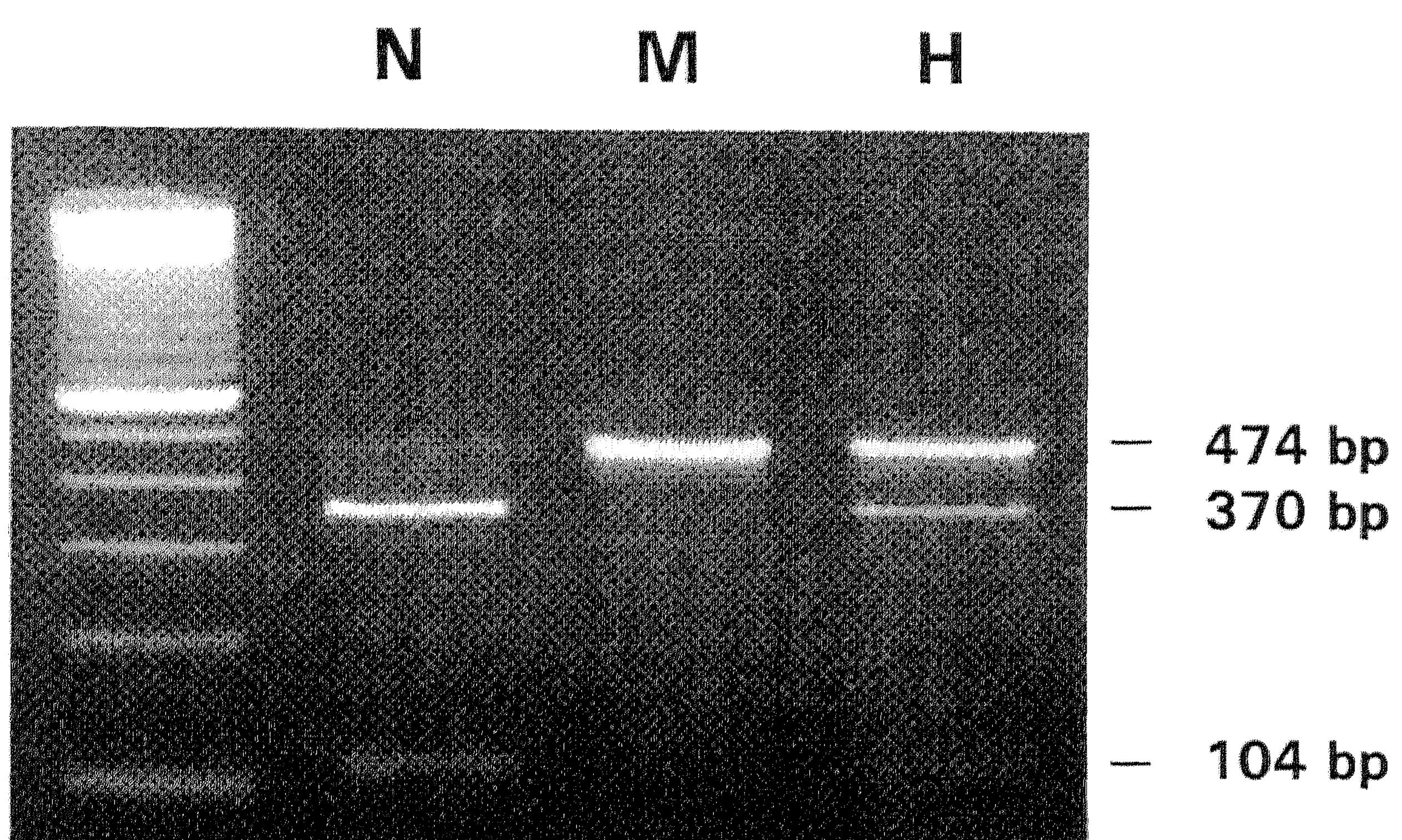


FIG. 2. Desmopressin infusion test in 7 NDI patients (\bullet) and 5 control (O). A tap-water load of 600 mL/m² was given orally, and basal urine was collected for an hour. Starting at time 0, desmopressin (Minirin^R, Ferring, Malmo, Sweden) was infused over 10 min in a dose of 0.3 μ g/kg. Urine and blood were collected at 0, 1, and 2 h. Mean \pm SD.

AQUAPORIN-2



tion. The parents, who tested to have normal urinary concentration, proved to be heterozygotes for the mutation. The unique feature of patients with an AQP2 defect is their selective renal resistance to vasopressin with intact responses of all other vasopressin target tissues. On random determinations the serum sodium and plasma osmolality of our patients were elevated, and consequential increased secretion of vasopressin can be assumed, though not determined in the present study. In that respect the high basal levels of the clotting factors may represent their response to chronic hyperstimulation by increased serum vasopressin levels. Yet, the response of the clotting factors to desmopressin was

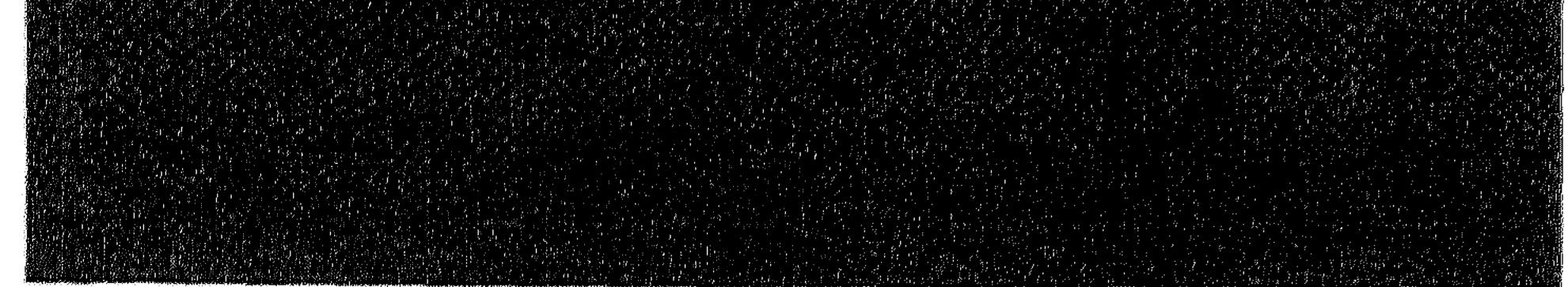


FIG. 3. MspI restriction digest analysis of the G298T mutation. The mutation resulted in the loss of an MspI restriction site in the first axon of the AQP2 gene. The region around the mutation was amplified by PCR, the product was digested with MspI, and the fragments were resolved on a 2% agarose gel and stained with ethidium bromide. For the mutant allele (M) the PCR product of 474 bp was not cleaved by MspI, whereas the wild type allele (N) gave two fragments of 370 bp and 104 bp. A parent of the patient shows three bands, indicative for heterozygosity. A marker is shown on the left lane.

similar to that of control subjects.

The differential response of clotting factors and urine osmolality to desmopressin may provide a simple tool for clinical diagnosis of a V_2 -postreceptor defect, where a molecular genetic expertise is unavailable. The only postreceptor defect reported so far is NDI caused by an autosomal recessive AQP2 defect, although on a theoretical basis, other postreceptor defects may be found in the future. The theoretical prospect that other, unknown, postreceptor defects may affect antidiuretic, but also coagulation factor response, leaves the molecular approach as the final diagnostic venue.

agarose gels, indicating heterozygosity for the G298T substitution (Fig. 3)

Discussion

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The present report describes autosomal inheritance of NDI in a cluster of 11 patients with an extremely high degree of consanguinity. The pedigrees and the normal urinary concentration of the parents suggested an autosomal recessive defect. The close proximity of their residences, within 20 km of each other, and the identical mutation identified in the index patients of families H and G suggest a common ancestor, although such an individual was not identified by history.

The G298T mutation in the AQP2 gene has not been described before. It results in a premature stop in protein translation in the third transmembrane region. If there would be any protein produced from this mutated gene, it could only encompass roughly a third of the protein missing from two of the three extracellular domains, two of the intracellular domains, as well as four of the transmembrane regions. As AQP2 belongs to a large family of transport proteins, in which the topology of six bilayer-spanning α helices connected by five loops is conserved in bacteria, yeast, plants, and mammals (12), it is difficult to imagine how such a truncated protein could exert its proper water channel func-

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