Nephrogenic Syndrome of Inappropriate Antidiuresis

in Adults: High Phenotypic Variability in Men and Women from a Large Pedigree

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Nephrogenic syndrome of inappropriate antidiuresis (NSIAD) is a recently described genetic cause of hyponatremia in male infants. Whether this X-linked condition could be detected in the adult or also could affect women is unknown. A large five-generation family was identified in which the recently described arginine-vasopressin receptor type 2 (AVPR2) mutation that is responsible for NSIAD was segregated. The proband was a 74-yr-old patient who had a syndrome of inappropriate antidiuresis and whose hyponatremia resisted administration of two AVPR2 antagonists. The phenotype of family members who carry the mutation was investigated. Patients with normal serum sodium were subjected to a water-load test. The previously reported activating missense R137C mutation in the AVPR2 gene in three hemizygous male and four heterozygous female individuals was identified. Except in one woman, spontaneous episodes of hyponatremia or abnormal water-load test were identified in all patients with the mutation, whether male or female. Skewed X inactivation was evidenced in the blood of the asymptomatic woman, which is compatible with preferential inactivation of her mutated allele. NSIAD is not limited to male infants. The diagnosis also should be considered in both male and female adults.


The syndrome of inappropriate antidiuresis is one of the most frequent causes of hyponatremia. In 10 to 20% of these patients, arginine-vasopressin (AVP) is reported to be undetectable (1). This suggests that inappropriate antidiuresis could be linked to mechanisms other than AVP hypersecretion. Recently, two cases of 3-mo-old boys with severe hyponatremia, inappropriate antidiuresis, and undetectable AVP levels were reported. A new mechanism that increases free water reabsorption was identified: A gain-of-function mutation in the AVP receptor type 2 (AVPR2) (2). The authors proposed the name of nephrogenic syndrome of inappropriate antidiuresis (NSIAD) to describe this new entity. The gene that encodes AVPR2 is located on the long arm of X chromosome (Xq28). Loss-of-function mutations of the same gene are responsible for X-linked nephrogenic diabetes insipidus (NDI; OMIM 304800) (3,4).

Although chronic syndrome of inappropriate antidiuresis is exceptional in children (a recent review of published cases in children between 1957 and 2004 reported only 13 cases, mostly associated with different brain abnormalities) (5), the first patients described with NSIAD were young male infants (2). NSIAD should follow an X-linked pattern, and because the AVPR2 is constitutively activated, heterozygote females are expected to show at least some degree of inappropriate antidiuresis. In the original study (2), the mother of one of the patients was carrying the same mutation and was found to have normal serum sodium (SNa), although no water-load test was performed.

During phase III clinical trials of two oral inhibitors of AVPR2 (tolvaptan and satavaptan) (6), we identified a 74-yr-old man who initially received a diagnosis of idiopathic syndrome of inappropriate secretion of antiuretic hormone (SIADH) and was not responding to these drugs. Upon sequencing of his AVPR2 gene, the previously described R137C mutation was found (2). We screened family members for the presence of the activating mutation and identified two additional hemizygous male and four heterozygous female individuals.

Our study demonstrates variable expressivity of NSIAD both in male and female individuals and highlights that activating mutation of AVPR2 might go unrecognized for years, until some variation in fluids and/or solute intake contributes to the development of hyponatremia. In one carrier woman, with normal SNa despite spontaneous high fluid intake, the pattern of X inactivation in peripheral blood demonstrated significant skewing.

Materials and Methods

Patients

We looked for activating mutation of the AVPR2 gene in the following patients:

Patient III.3 is the index patient (Figure 1). At the time of the study, he was 74 yr of age and was treated with urea (30 g/d) for 5 yr for hypona-
tremia considered to be secondary to idiopathic SIADH. He agreed to participate in two different phase III trials with tolvaptan (OPC-41061) and later satavaptan (SR121463B), both AVPR2 antagonists.

Patient V.9 is the grandson of patient III.3. He was born preterm at 32 wk of gestation with a birth weight of 1900 g. He presented perinatal anoxia and cerebral hemorrhage and developed hydrocephaly that justified establishment of a ventriculoperitoneal shunt.

Patient III.4 is the first cousin of patient III.3. At the time of this study, he was a 72-yr-old man with no history of hyponatremia. No information on the first years of his life was available. He reported never drinking more than 1.5 L/d. A water-load test (20 ml/kg in 15 min) was performed. Chest x-rays were normal.

Patient IV.5 is the daughter of patient III.3 and the mother of patient V.9. At the time of this study, she was 48 yr of age. There is no mention of inappropriate antidiuresis in her medical record. SNa was measured on three occasions over a period of a few months, and water-load test (20 ml/kg in 15 min) was performed.

Patient IV.7 is the daughter of patient III.4. She was 41 yr of age at the time of the study and followed a diet with 2.5 to 3.0 L/d of water intake, combined with a high regimen of fruits and vegetables. She reported episodes of dizziness with light headaches.

Patient IV.9, sister of patient IV.7, and her daughter, patient V.15, were 47 and 28 yr of age, respectively, at the time of the study. Patient IV.9 reported drinking spontaneously 3 L/d. Her SNa was measured. Water-load test was performed for patient IV.9.

Patients III.1, 2, 8, and 9 and V.6, 7, 8, 10, 11, and VI.14 presented no mutation and had no peculiar medical records relevant for this study. None of these patients was taking drugs that are known to induce hyponatremia or were known to present an endocrinopathy.

Water-Load Test

After baseline blood and urine samples were taken, 20 ml of water per kg of body wt (ml/kg BW) was given in 15 min to fasting patients. Urine samples were collected every 60 min for 240 min. A blood sample was taken at 240 min. An abnormal water-load test is defined as the inability to excrete at least 90% of the water within 4 h or failure to dilute urinary osmolality to values lower than 100 mOsm/kg H2O (7).

Usual blood and urine analyses were performed by the hospital laboratory.

Mutation Analysis

The AVPR2 mutation was detected by the following procedure. DNA was extracted from peripheral blood using standard procedures. All coding portions and intervening sequences of the AVPR2 gene were sequenced using two overlapping fragments amplified by PCR. The fragment that encompassed the mutation that was identified in this family was generated using forward primer 5'AGGGGAGTTCTGCGTGTCTG-3' and reverse primer 5'CCACACACACACATAGCG-3'.

PCR was performed in the GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA) in a 50-μl final volume that contained 250 ng of genomic DNA, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1 mM MgCl2, 0.01% gelatin, 200 μM dNTP, 0.05 U/μl Taq polymerase (Taq-Invitrogen, Merelbeek, Belgium), and 150 nM of each primer. The following conditions were used: Initial denaturation at 93°C for 2 min, then 35 cycles of 93°C for 1 min, 55°C for 1 min, and 72°C for 2 min, then 30 s, then 35 cycles of 93°C for 1 min, 55°C for 1 min, and 72°C for 2 min, then 30 s, and then 30 s and a final extension of 6 min. PCR product was purified with the Qiaquick PCR purification kit (Qiagen, Wetsburg, The Netherlands) and sequenced using the ABI PRISM Dye Terminator cycle sequencing Ready Reaction kit v1.1 (Applied Biosystems) according to the manufacturer’s instructions. Sequencing products were analyzed on a 3130xl Genetic Analyser sequencing machine (Applied Biosystems). The in silico mutation search was performed using the SeqScape software v2.5 (Applied Biosystems).

X-Chromosome Inactivation Analysis

The HUMARA locus (human androgen-receptor gene) on X-chromosome contains two methylation-sensitive HpaII restriction sites and is 90% polymorphic in white females for varying allele sizes (8). Analysis of X-inactivation status at the HUMARA locus was performed according to Allen and et al. (8) with slight modifications.

One micromgram of DNA was digested overnight at 37°C with 20 U of the restriction enzyme HpaII (New England Biolabs, Beverly, MA) in a 20-μl reaction; then both digested and undigested DNA samples were

Figure 1. Family tree illustrating transmission of the mutated arginine-vasopressin receptor type 2 (AVPR2) gene that is responsible for nephrogenic syndrome of inappropriate antidiuresis (NSIAD) over five generations. Mutation was tested and was absent in patients represented by a thicker outline. Obligate carriers who were not tested are represented by a hatched symbol. Heterozygous females are represented by a split symbol. Hemizygous males are represented by a full darkened symbol. Number within the symbol indicates the number of siblings of the same sex. The index patient is III.3.
amplified with oligonucleotide primers that flanked the polymorphic CTG repeat with exon 1 of the HUMARA gene. Four microliters of the restricted products or 200 ng of undigested DNA was subjected to PCR on a GeneAmp PCR System 9700 (Applied Biosystems) in GeneAmp PCR Buffer II (Applied Biosystems) supplemented with 2 mM Mg2+, 300 μM 7-deza-2'-deoxyguanosine 5'-triphosphate (Amersham Biosciences), 200 μM dATP, 200 μM dTTP, 200 μM dCTP, 50 μM dGTP, and 10% DMSO using 0.75 U of AmpliTaq Gold (Applied Biosystems) in a total reaction volume of 15 μL.

Primer sequences were 5'-TCCAGAATCTTGTTCCAGACGTGC-3' and 5'-GGCTGTGAAGGTGTTGCAGTTC-3'. The former primer was 5' labeled by 6-FAM fluorochrome. Cycling conditions were as follows: Initial denaturation step of 95°C for 3 min followed by 26 cycles of denaturation at 95°C for 1 min, annealing at 63°C for 2 min, and extension at 72°C for 1 min 30 s, followed by a final extension step at 72°C for 30 min.

PCR products were analyzed on a 3130xl Genetic Analyser sequencing machine (Applied Biosystems). Quantification was performed using the peak heights values defined by the GeneMapper software v3.5 (Applied Biosystems). The peak heights values corresponding to the two alleles in each female carrier. Factor VIIIc and antigenic Von Willebrand factor were measured in all of the patients.

All of the patients gave informed consent to genetic study. Phase III clinical trials were approved by the local ethic committee.

**Results**

Mutation analysis revealed c.409 C > T (NM_000054.2) transition, changing arginine to cysteine at codon 137 (R137C) in the AVPR2 gene of patients III.3, III.4, IV.5, IV.7, IV.9, V.9, and V.15. Patient III.3, the index patient, and his daughter's son, patient V.9, had a long-lasting history of inappropriate antidiuresis with recurrent episodes of symptomatic hyponatremia. Early in life, patient III.3 had mild growth deficiency, and during World War II, he had events that were compatible with seizures. SNa was not measured. The first episodes of hyponatremia were recognized at approximately the age of 60 yr (SNa of 131 mmol/L observed in 1991). Waterrestriction (<1.5 L/d) was effective in maintaining a normal SNa, but intermittent episodes of water intoxication were observed before treatment with urea was initiated (lowest SNa observed 114 mmol/L). Treatment with urea was initiated after a fall that was associated with SNa of 124 mEq/L, with osmolality of 256 mOsm/kg H2O. During the 5 yr of treatment with urea, SNa was measured approximately 50 times and mostly was >132 mmol/L (mean 134 ± 4 mmol/L). The patient could monitor his treatment easily according to morning body weight. During phase III trials, despite a progressive increase in the daily dose of AVPR2 antagonists (from 15 to 60 mg/d for tolvaptan and a full dosage of 50 mg/d for satavaptan), no efficacy on urine dilution capacity was observed (Figure 2). Table 1 summarizes blood and urine parameters during liberal water intake, water restriction (<1.5 L/d), treatment with urea (30 g/d) with liberal water intake, treatment with tolvaptan (60 mg/d), and treatment with satavaptan (50 mg/d). Chest x-ray was normal.

Patient V.9 had first episodes of hyponatremia at 7 mo of age (Table 2). At 16 d of age, urine osmolality was 94 mOsm/kg with a SNa of 135 mmol/L. First episodes of hyponatremia were recognized when he was 7 mo of age and were attributed to SIADH secondary to his brain lesions. Salt supplementation (2 to 6 g/d) was initiated but did not protect him from frequent episodes of hyponatremia (lowest value 116 mmol/L), unless water restriction was respected. At the time of this study, patient V.9 was 24 yr of age and taking 4 g of salt tablets daily, combined with mild water restriction (<1.5 L/d). He had mild mental retardation with right hemiparesis. Salt tablets were replaced by urea (15 g/d urea increased to 30 g/d after 1 mo). Both patients benefited from daily administration of 30 g of urea. Under this medication, it is interesting to note that in patient V.9 BP was lower and that high AVP level was observed in patient III.3 (Table 1).

Patient III.3, the cousin of patient III.3, was a 72-yr-old man without history of hyponatremia. At the start of the water-load test, his urine was highly concentrated (792 mOsm/kg H2O) despite low ADH levels (1.1 pg/ml). There was no decrease in urine osmolality or increase in diuresis despite the presence of mild hyponatremia (SNa 132 mEq/L) at the end of the test (Figure 3). This shows clearly that the patient was unable to dilute his urine despite high fluid intake.

Patient IV.5, the mother of patient V.9, was heterozygous for the R137C mutation. Although she had no complaint, baseline SNa was measured on three occasions over period of few months and was normal. The water-load test showed that she was able to increase her diuresis (from 20 to 80 ml/h) and decrease her urine osmolality (from 820 to 417 mOsm/kg H2O), but these values clearly are abnormal; only 18% of the water load was eliminated within 4 h (Figure 3).

Patient IV.7 was dieting with high fluid intake (25 to 3 L/d) combined with a high intake of fruits and vegetables. The patient presented hyponatremia (SNa 124 mEq/L; serum osmolality 270 mOsm/kg H2O) with a urine osmolality of 432 mOsm/kg H2O and very low ADH levels (0.6 pg/ml). SNa was normalized after she kept her fluid intake below 2 L/d (Table 3).

Patient IV.9, the sister of patient IV.7, did not present a history of hyponatremia, but the water-load test showed clearly...
abnormal value: Only 20% of the water load was eliminated within 4 h, with a minimum urine osmolality of 513 mOsm/kg H2O and a SNa of 133 mEq/L at the end of the test (Figure 3).

Patient V.15, although she is heterozygous for the mutation and reportedly drinks spontaneously /H11022 3 L/d, presented with a normal SNa (143 mmol/L) and a urine osmolality of 250 mOsm/kg. To explore this discordance between her genotype and phenotype, a study of the pattern of X inactivation was performed. Patient V.15 displayed clear skewing of her X-inactivation pattern. In comparison, the pattern was symmetrical, as expected, for all other female patients (Figure 4).

Discussion

R137C mutation in AVPR2 gene was described recently in an infant with NSIAD (2). This mutation affects the highly conserved "DRY/H" motif that is typical of class 1 G protein–coupled receptors. As in other rhodopsin-like G protein–coupled receptors (9), mutation of the conserved arginine was shown to confer constitutive activity to the receptor, as demonstrated by its ability to increase cAMP levels in transiently transfected heterologous

### Table 1. Some clinical and biological data in three adult men with NSIAD under different treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Patient III.3 (74 yr of age)</th>
<th>Patient V.9 (21 yr old)</th>
<th>Patient V.9 (24 yr old)</th>
<th>Patient III.4 (73 yr old)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LW (entar 1.5 L/d)</td>
<td>WR (entar 3 g/d)</td>
<td>NaCl (2 g/d)</td>
<td>NaCl (4 g/d)</td>
<td>Urea (15 g/d)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>70</td>
<td>69</td>
<td>69</td>
<td>68.5</td>
<td>68.5</td>
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<tr>
<td>BP (mmHg)</td>
<td>120/70</td>
<td>127/75</td>
<td>140/90</td>
<td>120/60</td>
<td>110/70</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>10</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>25&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.9</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>4</td>
<td>3.7</td>
<td>4.6</td>
<td>4.5</td>
<td>5.5</td>
</tr>
<tr>
<td>SNa (mmol/L)</td>
<td>124</td>
<td>134</td>
<td>129</td>
<td>131</td>
<td>140</td>
</tr>
<tr>
<td>ADH (pg/ml)</td>
<td>1.2</td>
<td>—</td>
<td>0.9</td>
<td>—</td>
<td>7</td>
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<tr>
<td>Standing aldosterone (pg/ml)</td>
<td>—</td>
<td>151</td>
<td>28</td>
<td>—</td>
<td>115</td>
</tr>
<tr>
<td>Standing renin (ng/l)</td>
<td>—</td>
<td>10.6</td>
<td>—</td>
<td>10</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>NTpro BNP (pg/ml)</td>
<td>—</td>
<td>—</td>
<td>10</td>
<td>—</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Urine volume (ml/24 h)</td>
<td>800</td>
<td>960</td>
<td>1.150</td>
<td>1.050</td>
<td>1.450</td>
</tr>
<tr>
<td>Urine urea (g/24 h)</td>
<td>15</td>
<td>19</td>
<td>20.5</td>
<td>20</td>
<td>41</td>
</tr>
<tr>
<td>Urine Na (mmol/L)</td>
<td>48</td>
<td>140</td>
<td>116</td>
<td>65</td>
<td>145</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td>615</td>
<td>768</td>
<td>690</td>
<td>625</td>
<td>802</td>
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</table>

<sup>a</sup>ADH normal value 1 to 7 pg/ml; standing aldosterone normal value 40 to 310 pg/ml; standing renin normal value 3 to 33 ng/L; NTpro BNP normal value <150 pg/ml. ADH, antidiuretic hormone; LW, liberal water intake; NTpro BNP, N-terminal natriuretic peptide type B; SNa, serum sodium; WR, water restriction;
<sup>b</sup>Data obtained after 5 d on 60 mg of tolvaptan.
<sup>c</sup>Data obtained after 5 d on 50 mg of satavaptan.
<sup>d</sup>Data obtained 2 h after urea intake.

### Table 2. Some clinical and biological data of the childhood period of patient V.9

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>16 d</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>1.95</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>8</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.5</td>
</tr>
<tr>
<td>SNa (mmol/L)</td>
<td>135</td>
</tr>
<tr>
<td>Urine volume (ml/24 h)</td>
<td>135</td>
</tr>
<tr>
<td>Urine urea (g/24 h)</td>
<td>1.25</td>
</tr>
<tr>
<td>Urine Na (mmol/L)</td>
<td>9</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td>94</td>
</tr>
</tbody>
</table>

abnormal value: Only 20% of the water load was eliminated within 4 h, with a minimum urine osmolality of 513 mOsm/kg H2O and a SNa of 133 mEq/L at the end of the test (Figure 3).

Patient V.15, although she is heterozygous for the mutation and reportedly drinks spontaneously >3 L/d, presented with a normal SNa (143 mmol/L) and a urine osmolality of 250 mOsm/kg. To explore this discordance between her genotype and phenotype, a study of the pattern of X inactivation was performed. Patient V.15 displayed clear skewing of her X-inactivation pattern. In comparison, the pattern was symmetrical, as expected, for all other female patients (Figure 4).
cells. In this study, we describe a 74-yr-old man and members of his family who harbor the same R137C activating mutation of AVPR2. The proband had a long-lasting history of inappropriate antidiuresis and symptomatic episodes of hyponatremia. He initially received a diagnosis of SIADH and was treated successfully by administration of 30 g/d urea. NSIAD was suspected because he showed no response to two different AVPR2 antagonists (tolvaptan and satavaptan) that were administrated during the course of phase III clinical trials: Urine remained concentrated despite hyponatremia together with low AVP values. Some of our patients with chronic SIADH treated with V2 antagonist did not show correction of their hyponatremia. Most of these patients likely presented a “reset osmostat.” After medication an increase of thirst and fluid intake is observed, with increased diuresis and reduced urine osmolality (10). This phenomenon was not observed in Patient III.3 (Figure 2).

Two additional male individuals of the family were hemizygous for the mutation and displayed different phenotypes. In Patient V.9 (the grandson of the proband), hyponatremia was noted at 7 mo and attributed to SIADH. It is known that the

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**Table 3.** Some clinical and biological data in four women with NSIAD (heterozygous) under variable water intake

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fluid Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient IV.7 (41 yr old)</td>
</tr>
<tr>
<td></td>
<td>H + D</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>75</td>
</tr>
<tr>
<td>BP (mmHg)</td>
<td>120/80</td>
</tr>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>10</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.7</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>2.5</td>
</tr>
<tr>
<td>SNa (mmol/L)</td>
<td>124</td>
</tr>
<tr>
<td>ADH (pg/ml)</td>
<td>0.6</td>
</tr>
<tr>
<td>Aldosterone (pg/ml)</td>
<td>169</td>
</tr>
<tr>
<td>Renin (ng/L)</td>
<td>&lt;2.5</td>
</tr>
<tr>
<td>NTpro BNP (pg/ml)</td>
<td>212</td>
</tr>
<tr>
<td>Urine volume (ml/24 h)</td>
<td>2100</td>
</tr>
<tr>
<td>Urine urea (g/24 h)</td>
<td>19.6</td>
</tr>
<tr>
<td>Urine Na (mmol/24 h)</td>
<td>157</td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td>432</td>
</tr>
</tbody>
</table>


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*D, slimming diet; H, high fluid intake 2.5 to 3 L/d; N, normal fluid intake <2 L/d. See Table 1 for normal values.*
was not addressed in the first report of NSIAD, penetrance of the
identified in this study showed some degree of inappropriate
know decrease in solute intake in the elderly (17,18).

It is not excluded that some of these patients in fact present
hand (15). Idiopathic chronic SIADH is frequent in the elderly (16);
differences and variation in fluid and/or solute intake on the other

ers, on one hand, and by the major environmental influence of
influence of modifier genes, as for many genetic dis-
relatively late in life. This variable expressivity could be explained
by the influence of modifier genes, as for many genetic dis-
edes, on one hand, and by the major environmental influence of
differences and variation in fluid and/or solute intake on the other

ability to concentrate urine increases slowly during the first
months of life, being maximal at 18 mo (11). In prematurity
(particularly when combined with asphyxia), urine is reported
to be mostly hypotonic (12). It is interesting that as a neonate,
despite the presence of the mutation, patient V.9 was able to
dilute his urine to a very low osmolality (94 mOsm/kg at day
16), thereby protecting him from episodes of hyponatremia. He
presented even transient hyponatremia (Table 2), a well
known complication of prematurity, partly related to the uri-
inary concentration deficit (13,14). Hyponatremia developed
only later, when his capacity to concentrate urine increased,
and finally was recognized at 7 mo. In the two previously
reported children with NSIAD, symptomatic hyponatremia
was noted at the age of 3 and 2.5 mo with reported urine
osmolality of 284 and 390 mOsm/kg, respectively (2).

The third hemizygous male individual was (apparently)
asymptomatic throughout life, until the water-load test unmasked
his inability to dilute his urine. This observation points to the
highly polymorphic trinucleotide CAGn repeat in exon 1 of the
HUMARA gene on the X chromosome and two HpaII restric-
tion sites. Informative samples show heterozygosity. This re-
peat, when methylated (inactive X), is resistant to HpaII diges-
tion. When unmethylated (active X), no PCR amplified material
is observed after HpaII digestion. Normally, equal proportions
of each X chromosome resist HpaII digestion (left). In the case
of skewing of X chromosome inactivation, one HUMARA allele
is preferentially digested (right, arrow).

Figure 4. PCR amplification before and after HpaII digestion in
a symptomatic patient (IV.5; left) and the asymptomatic patient
V.15 (right). Primers used in the HUMARA assay amplify the
highly polymorphic trinucleotide CAGn repeat in exon 1 of the
HUMARA gene on the X chromosome and two HpaII restric-
tion sites. Informative samples show heterozygosity. This re-
peat, when methylated (inactive X), is resistant to HpaII diges-
tion. When unmethylated (active X), no PCR amplified material
is observed after HpaII digestion. Normally, equal proportions
of each X chromosome resist HpaII digestion (left). In the case
of skewing of X chromosome inactivation, one HUMARA allele
is preferentially digested (right, arrow).

disease in female individuals is logically expected for a gain-of-
function mutation located on the X chromosome. This contrasts
with the typical recessive transmission of X-linked NDI, whereby
female individuals who carry the inactivating mutation of AVPR2
mostly are reported to be asymptomatic (3,4,19). Indeed, under
unbiased X inactivation, approximately half of the epithelial cells
of the collecting duct of the kidney will have a constitutively active
AVPR2, resulting in circumstances of high fluid intake in an
inappropriate antidiuresis. One the four heterozygous female in-
dividuals (V.15) was asymptomatic. She presented normal natre-
mia and an appropriate urine osmolality of 250 mOsm/kg for the
high fluid intake that she reported. It is interesting that this
woman is the only one in the pedigree to show a skewed pattern
of X inactivation. Skewed X inactivation has been reported as a
mechanism to explain that female patients who are heterozygous
for an AVPR2 inactivating mutation may experience NDI (19,20).
Whereas preferential inactivation of the normal allele accounts for
these rare cases of NDI in female individuals (19), in this case, one
has to postulate preferential inactivation of the mutant allele to
explain the normal phenotype of patient V.15.

Treatment of NSIAD poses a challenge, especially in infancy,
because fluid restriction is associated with limited calorie intake.
In both symptomatic patients, urea was an efficient and conve-
nient medication to induce osmotic diuresis. It also was the med-
ication of choice for the two previously reported infants (2,5).
The ineffectiveness of the two AVPR2 antagonists tested in the index
patient was the lead to the diagnosis of NSIAD in this family. This
is interesting, both from a clinical and a fundamental point of
view. Clinically, it suggests that administration of AVPR2 antag-
onists may provide a diagnostic tool for the selection of patients
who need sequencing of their receptor gene. Fundamentally, it
illustrates that at least some of the currently tested AVPR2 antagon-
ists are not endowed with inverse agonist properties that are
able to function as an inverse agonist on a consti-
tructively active AVPR2 mutant made by site-directed mutagenesis.
This mutant is expected to be very similar structurally to R137C,
because its amino acid substitution affects the immediately up-
stream aspartic acid of the DRY/H motif (D136A) (21,22). Also,
the naturally occurring AVPR2 mutant made by site-directed mutagenesis.
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because its amino acid substitution affects the immediately up-
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the naturally occurring AVPR2 mutant made by site-directed mutagenesis.
This mutant is expected to be very similar structurally to R137C,
because its amino acid substitution affects the immediately up-
stream aspartic acid of the DRY/H motif (D136A) (21,22). Also,

Conclusion
The prevalence of activating mutation of AVPR2 is unknown.
Because as many as 10% of patients with SIADH have undetect-
able levels of AVP (1), activating mutations of AVPR2 are likely to
account for at least some of these cases. However, one family was
described with no mutation in the coding exons and flanking
introns of AVPR2 gene (26), which suggests that other

Figure 4. PCR amplification before and after HpaII digestion in
a symptomatic patient (IV.5; left) and the asymptomatic patient
V.15 (right). Primers used in the HUMARA assay amplify the
highly polymorphic trinucleotide CAGn repeat in exon 1 of the
HUMARA gene on the X chromosome and two HpaII restric-
tion sites. Informative samples show heterozygosity. This re-
peat, when methylated (inactive X), is resistant to HpaII diges-
tion. When unmethylated (active X), no PCR amplified material
is observed after HpaII digestion. Normally, equal proportions
of each X chromosome resist HpaII digestion (left). In the case
of skewing of X chromosome inactivation, one HUMARA allele
is preferentially digested (right, arrow).
molecular mechanisms of NSIAD still have to be unraveled. Our study demonstrates that NSIAD displays a wide variation of expressivity. It is not limited to male infants, and the diagnosis also should be considered in both male and female adults.

Acknowledgements
This study was supported by grants form the Fonds National de la Recherche Scientifique, conventions 3.4509.03 and 3.4.552.05 to G.D. and G.V., respectively. Also supported by the Belgian Program of Interuniversity Poles of Attraction (IUAP/PAI P5/30) and the Erasme Fund and the LifeSciHealth program of the European Community (grants LSHB-CT-2003-503337 and LSHB-CT-2004-518167).

We thank Dr. P. Lewalle for referring the index patients, Dr. M. Abramowicz for constructive discussion, and Prof. A. Persu (UCL en Wolluwe) and Prof. S. Vanderschueren (KOL, Leuven) for helping to

Grant Support
This study was supported by grants from the Fonds National de la Recherche Scientifique, conventions 3.4509.03 and 3.4.552.05 to G.D. and G.V., respectively. Also supported by the Belgian Program of Interuniversity Poles of Attraction (IUAP/PAI P5/30) and the Erasme Fund and the LifeSciHealth program of the European Community (grants LSHB-CT-2003-503337 and LSHB-CT-2004-518167).

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