A Novel Mutation in the Vasopressin V2 Receptor Gene in a Woman with Congenital Nephrogenic Diabetes Insipidus

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A 56-year-old Japanese woman with congenital nephrogenic diabetes insipidus (CNDI) is reported. She was diagnosed with CNDI accompanied by advanced gastric cancer. After total gastrectomy, approximately 500 ml fluid per hour was necessary to prevent dehydration. Urinary volume was decreased by administration of hydrochlorothiazide. We detected a novel mutation in the vasopressin V2 receptor gene of her chromosomal DNA. A substitution from G to A was found at the 631 nucleotide position, altering codon 12 from glycine (GGG) to glutamic acid (GAG) in the first extracellular domain. This missense mutation appeared to be the cause of her resistance to arginine vasopressin. (Internal Medicine 38: 808-812, 1999)

Key words: polyuria, vasopressin receptor, missense mutation

Introduction

Congenital nephrogenic diabetes insipidus (CNDI) is usually an X-linked recessive disease, characterized by renal resistance to the anti-diuretic effect of arginine vasopressin (AVP). AVP binds to specific receptors on renal collecting tubule cells, and a signal is transmitted by interaction with membrane-associated proteins, leading to the production of cyclic adenosine monophosphate (cAMP). Elevation of the intracellular cAMP level increases water permeability via expression of water channels on cell membranes through the activation of protein kinase-A (1). Thus, any defect in these processes might be a cause of CNDI. Human vasopressin V2 receptor gene was cloned in 1992 (2, 3), and distinct mutations in this receptor gene have since been found in CNDI families (4-12).

We describe here a novel mutation identified in a Japanese female with this disorder, a missense mutation from Gly to Glu (GGG to GAG) at the 12th codon in the first extracellular domain of the vasopressin V2 receptor gene.

For editorial comment, see also p 755.

Case Report

A 56-year-old Japanese woman was admitted to our hospital because of palpitation and vertigo. A diagnosis of severe anemia was made. To elucidate the cause of the anemia, gastroendoscopic examination was performed, and revealed advanced gastric cancer at the greater curvature. Physical examination revealed a normally grown and slightly thin woman. Her height was 153.5 cm, weight 45.0 kg, temperature 36.5°C, pulse 84 beats/min and regular, and blood pressure 114/64 mmHg. The lung, heart, abdomen, and extremities were normal. The laboratory data on admission are presented in Table 1. Urinary examination was normal, but hemoglobin level and number of erythrocytes were very low, indicating severe hypochromic anemia. The serum sodium was 150 mEq/l, potassium 4.0 mEq/l, chloride 114 mEq/l, calcium 8.8 mg/dl, and phosphorus 3.5 mg/dl. Blood urea nitrogen concentration was 6 mg/dl, and creatinine 0.5 mg/dl.

To examine for metastasis of gastric cancer, we examined the abdomen by using computed tomography, which revealed marked bilateral hydronephrosis. We determined her daily urine volume to be 13,200 ml/day with a specific gravity of 1.004. Since childhood she had drunk approximately 10 liters or more of water per day due to thirst. We therefore suspected diabetes insipidus.

A water deprivation test was performed (Fig. 1). After 5 hours of water deprivation, urinary osmolality was only 44 mOsm/l, while the plasma osmolality rose from 274 to 305 mOsm/l, and the patient’s weight decreased by 3.0 kg. It was 6.7% of body weight which was measured just before the water deprivation test. These findings indicated diabetes insipidus. Nasal administration of 1-desamino-8-d-arginine vasopressin (DDAVP) was started with an initial daily dose of 10

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Mutation in Vasopressin V2 Receptor Gene

Table 1. Laboratory Data

<table>
<thead>
<tr>
<th>Urinalysis</th>
<th>Hematology</th>
</tr>
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<tbody>
<tr>
<td>PH</td>
<td>Red blood cell</td>
</tr>
<tr>
<td>5</td>
<td>179×10^9/μl</td>
</tr>
<tr>
<td>Protein (-)</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>Sugar (-)</td>
<td>4.5 g/dl</td>
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<tr>
<td>Blood (-)</td>
<td>Hematocrit</td>
</tr>
<tr>
<td>16%</td>
<td>52.1×10^9/μl</td>
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<tr>
<td>Casts (-)</td>
<td>Platelet</td>
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<tr>
<td></td>
<td>5.570/μl</td>
</tr>
<tr>
<td>ESR</td>
<td>35 mm/h</td>
</tr>
</tbody>
</table>

Blood Chemistry

| GOT 14 IU/l          | Na 150 mEq/l             |
| GPT 13 IU/l          | K 4.0 mEq/l              |
| LDH 347 IU/l         | Cl 114 mEq/l             |
| γ-GTP 11 IU/l        | Ca 8.8 mg/dl             |
| Total bilirubin      | P 3.5 mg/dl              |
| 0.3 mg/dl            | Fe 25 μg/dl              |
| Total protein        | UIBC 452 μg/dl           |
| 6.9 g/dl             | BUN 109 mg/dl            |
| Albumin 3.3 g/dl     | FBS                       |
| 0.5 mg/dl            | HbA1c 4.1%               |


![Figure 1. Water deprivation test. The patient failed to concentrate her urine despite increase in plasma osmolality.](image)

μg, however, urine volume was not decreased, so the daily dose of this agent was finally increased to 30 μg, still without reduction of daily urine volume. After one week, the plasma AVP level was as high as 10 pg/ml and increased to 22 pg/ml after a 5-hour water deprivation test.

She was diagnosed with CNDI on the basis of clinical manifestations including a lifelong history of polyuria and polydipsia, elevated plasma concentrations of AVP, and unconcentrated urine despite water deprivation and exogenous administration of DDAVP. At 28th day after admission, total gastrectomy was performed. Unfortunately, the gastric cancer had disseminated throughout the peritoneum. Postoperatively, about 500 ml fluid per hour was required to prevent dehydration, referring to an hour urine volume, central venous pressure, and plasma osmol-
lality. She was therefore treated with a low-solute concentrated diet and a thiazide diuretic (hydrochlorothiazide) (13). Serum electrolyte levels returned to normal and urine volume decreased to 3,000-4,000 ml/day (Fig. 2).

Direct dideoxy-DNA sequencing was performed for the entire coding region of the human vasopressin V2 receptor gene. Direct sequencing analysis revealed that the patient had a G-to-A transition at nucleotide position 631, altering codon 12 from GGG to GAG in the first extracellular domain (Figs. 3, 4).

Discussion

CNDI is a rare disease characterized by polydipsia and polyuria with low urine specific gravity from soon after birth. Unless recognized and treated early, persistent severe dehydration may lead to growth retardation and mental retardation, or death (14).

The present patient had consistently drank more than 10 liters of water per day because of thirst, therefore had no mental retardation and an average intelligence, and in addition had two healthy children. They exhibited no CNDI symptoms.

Since CNDI is generally transmitted as an X-linked recessive disorder, the clinical disorder occurs primarily in males (15). The patient’s father died at age 31 of pulmonary tuberculosis, and exhibited no CNDI symptoms. Her mother also had no history of polyuria and polydipsia and did not have a mutation in the vasopressin V2 receptor gene. Thus, in this case the mutated gene was not inherited from her parents. She was heterozygous for this mutation, as a result of the random X chromosome inactivation (16, 17). She has a clinical form of CNDI. The human V2 receptor gene is located in the region of chromosome Xq 28 (18) and has three exons and two small introns (3). The cDNA sequence predicts a polypeptide of 371 amino acids with a structure typified by guanine nucleotide (G), with seven transmembrane, four extracellular and four cytoplasmic domains (2, 3). More than 70 V2 receptor gene mutations have been detected in CNDI families to date, including missense
mutations (9, 10, 12), deletions (4, 6, 8), insertions (7, 11), and nonsense mutations (5, 9, 10) leading to a truncated receptor.

Holtzman et al reported a nonsense mutation at codon 231 resulting from a G-to-T transition (11). This mutation causes premature termination and truncation of the receptor protein, which then contains only 231 amino acids instead of the normal 371. This protein potentially spans the cell membrane only five times instead of the usual, and lacks the entire carboxyl terminus of the receptor protein. Other nonsense mutations such as Q119X (5), L312X (12), and R337X (10) have also been reported. These nonsense mutations did not produce complete receptor proteins, and resulted in nonfunctional proteins. One type of deletion changes codon 246 from GGG to GGC, shifts the reading frame for protein translation (7). As a consequence of this change in the amino acid sequence, 40 percent of the receptor sequence at the carboxyl terminus was disrupted, first by the generation of a missense amino acid sequence and then by premature termination.

The resulting mutant receptor thus lacks the entire carboxyl-terminal third of the normal protein and retains only 17 of the amino acids of the third intracellular loop. The insertion of a single cytosine into the codon for leucine 228 in the V2 receptor sequence shifted the reading frame for protein translation (7). As a consequence of this change in the amino acid sequence, 40 percent of the receptor sequence at the carboxyl terminus was disrupted, first by the generation of a missense amino acid sequence and then by premature termination.

The human V2 receptor has two cysteine residues (Cys 112 and Cys 192, in the second and third extracellular domains, respectively) which are conserved in the vasopressin receptor, suggesting that they are required for structural or functional integrity of the receptor (19). An arginine to cysteine missense mutation was found at codon 181 (5). It is thus possible that this Arg to Cys substitution disrupts the correct disulfide binding between extracellular loop I and II. In other cases, missense mutations such as Gly 185 to Cys, Tyr 205 to Cys, and Arg 203 to Cys have been reported (20). It is conceivable, then, that these extra cysteines interfere with the formation of disulfide bridges, which may affect vasopressin binding. Holtzman et al reported a missense mutation, arginine to tryptophan at codon 113 (21). They speculated that a change from a positively charged arginine residue to an amino acid with a large side chain with both hydrophobic and hydrophilic characteristics such as tryptophan, might interfere with the formation of disulfide bonds, thereby rendering the receptor dysfunctional because it remains trapped in intracellular organelles. The R137H mutant receptor exhibited unaltered binding affinity for vasopressin, compared to the wild type, but failed to stimulate the adenylate cyclase system (22).

In the present study, a direct sequencing analysis revealed her genomic DNA has a missense mutation from G to A at the 631st nucleotide in an allele of vasopressin V2 receptor gene. The resulting 12th codon is substituted from glycine (GGG) to glutamic acid (GAG) by the missense mutation in the extracellular domain of vasopressin V2 receptor gene. The substitution leads to a change in the three-dimensional structure of the
V2 receptor and decreases the binding affinity for vasopressin protein, since the polarity change of amino acid is lead by the substitution from the neutral amino acid to the acidic amino acid. This mutation was considered as the cause of CNDI in this case.

References