Correlation between Clinical Phenotypes and X-inactivation Patterns in Six Female Carriers with Heterozygote Vasopressin Type 2 Receptor Gene Mutations

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Abstract. About 90% of patients with congenital nephrogenic diabetes insipidus (NDI) have vasopressin type 2 receptor (V2R) gene mutations that are inherited in an X-linked recessive manner. Although most female carriers are asymptomatic, some female carriers show polydipsia and polyuria. The reason why female carriers show NDI symptoms is explained by skewed X-inactivation. We studied X-inactivation patterns of six female carriers with heterozygote V2R gene mutations. The X-inactivation pattern in peripheral blood leukocytes was examined using methylation analysis of the polymorphic CAG repeat in the androgen receptor gene. Two asymptomatic female carriers showed random X-inactivation (61.9% and 60.7%). Skewed X-inactivation patterns (71.6%, 79.4%, and 91.2%) occurring preferentially to normal X alleles were recognized in three female carriers who showed clinical NDI symptoms. However, in one female carrier who showed clinical NDI symptoms, random X-inactivation (55.4%) was recognized. In conclusion, the clinical NDI phenotypes may correlate with the X-inactivation patterns in female carriers with heterozygote V2R gene mutations. However, in some female carriers, we cannot predict the clinical phenotypes by the evaluation of the X-inactivation patterns in peripheral blood leukocytes, because X-inactivation ratios within an individual are sometimes different between tissues.

Key words: Nephrogenic diabetes insipidus, Vasopressin receptor, X-inactivation, Female carrier

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ABOUT 90% of patients with congenital nephrogenic diabetes insipidus (NDI) are males who have mutations of the vasopressin type 2 receptor (V2R) gene [1] on the X chromosome (Xq28). The remaining 10% patients have mutations of the aquaporin 2 (AQP2) water channel gene [2] on chromosome 12q13, which are inherited in an autosomal recessive manner. Although most of the female carriers with heterozygote V2R gene mutations are usually asymptomatic, some female carriers have been reported to show polydipsia and polyuria similar to those of male patients [3–7]. The reason why female carriers with heterozygote V2R gene mutations show NDI symptoms is explained by skewed X-inactivation [5–7]. We studied the correlation between the clinical NDI phenotypes and the X-inactivation patterns in six female carriers with heterozygote V2R gene mutations.

Subjects and Methods

Subjects

Family 1

The proband was a 20 year old male. He was initially referred to another hospital because of fever on the 11th day of his life, and was diagnosed as having congenital NDI. When he was six years old, he was brought to our hospital and was reevaluated. At that time, his urine osmolality showed little increase, both
after the water deprivation test (before: 75 mOsm/kg, and after: 89 mOsm/kg) and after an infusion of 20 µg desmopressin acetate (DDAVP) (before: 61 mOsm/kg, and after: 71 mOsm/kg). His mother was asymptomatic. The family tree of Family 1 is shown in Fig. 1-a.

**Family 2**

The proband was a 9 year old girl. She was referred to our hospital because of polydipsia (3 liters/day; 5 liters/m²/day) when she was 3 years old. Since the change in her urine osmolality before and after the water deprivation test was small (before: 86 mOsm/kg, and after: 130 mOsm/kg) and the intramuscular infusion of 2 U pitressin did not concentrate her urine osmolality sufficiently (before: 86 mOsm/kg, and after: 216 mOsm/kg), she was diagnosed as having congenital NDI. Her father also showed polydipsia (8–9 liters/day) and polyuria. The family tree of Family 2 is shown in Fig. 1-b.

**Family 3**

The proband was a 6 year old boy. He received a diaphragmatic relaxation operation on the 5th day of the life. After the operation, hypernatremia (150–160 mM) was recognized and his plasma arginine vasopressin (AVP) was extremely high (26.0 pg/ml). Since the infusion of 2.5 µg desmopressin acetate (DDAVP) did not increase urine osmolality (before: 60 mOsm/kg, and after: 100 mOsm/kg), he was diagnosed as having congenital NDI. His mother also showed polydipsia (4 liters/day) and polyuria. The family tree of Family 3 is shown in Fig. 1-c.

**Family 4**

The proband was a 1 year old boy. He was referred to the hospital because of fever when he was 1 month old. His serum sodium level was high (152–166 mM) and polyuria (600–900 ml/day: 3–4.5 liters/m²/day) was recognized. Since the infusion of desmopressin acetate (DDAVP) did not increase urine osmolality, he was diagnosed as having congenital NDI. His maternal uncle had been diagnosed as having congenital NDI in infancy. His mother and maternal grandmother also showed polydipsia (3–4 liters/day and 5–6 liters/day, respectively) and polyuria. However, his maternal aunt was asymptomatic. The family tree of Family 4 is shown in Fig. 1-d.

The clinical findings of six female carriers (the mother in Family 1, the proband in Family 2, the mother in Family 3, the mother, the aunt and the grandmother in Family 4) are shown in Table 1.

**Methods**

The study was approved by the institutional review board (Toho University School of Medicine), and informed consent to participate in the study was obtained from patients’ parents or each subject. Genomic DNA was extracted from peripheral blood leukocytes. All exons of the V2R gene were amplified by polymerase chain reaction (PCR). Primers for amplifying V2R gene are shown in Table 2. The genomic DNA was amplified using two sets of primers (F1-R2, F2-R4). The PCR fragment produced using primer pair F1-R2 was then amplified with nested primer pair F1-R1, and the PCR fragment produced using primer pair F2-R4.
was amplified with five sets of nested primer pairs (F2-R1, F3-R2, F4-R3, F5-R3, and F6-R4). The PCR conditions were 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C for 35 cycles. Each amplified product was gel purified and sequenced by an automated DNA sequence analyzer (ABI 310 automatic sequencer, Applied Biosystems, Foster City, CA).

The X-inactivation patterns of female carriers were investigated by studying the polymorphic trinucleotide (CAG) repeat in the first exon of the human androgen receptor gene [8]. First, DNA samples were digested using methylation-sensitive restriction enzymes (HpaII and HhaI) whose cleavage sites were close to the CAG repeat element in the first exon of the androgen receptor gene. Both digested and undigested DNA samples were amplified by PCR method using primers for the androgen receptor locus that included the HpaII and HhaI cleavage sites and the CAG repeat element, as reported in Allen et al. [8]. At that time, the forward primer was modified with fluorescein (6'-FAM) [9]. In digested samples, amplification occurred if the restriction sites were methylated, and amplification did not occur if the restriction sites were not methylated. Next, the PCR products were resolved by electrophoresis, with Rox molecular markers, in an automated sequencer, and peak heights were analyzed using GeneScan software (Applied Biosystems). Peak height ratios for the two alleles in digested samples were corrected using peak height ratios for the two alleles in undigested samples. The ratios of the skewed X-inactivation in digested samples were then calculated by normalizing the sum of the two alleles to 100%.

The X-inactivation pattern was classified as random (allele ratios 50 : 50–65 : 35), moderately skewed (ratios 65 : 35–80 : 20), highly skewed (ratios 80 : 20–90 : 10) and extremely skewed (ratios 90 : 10–100 : 0), following the classification used in previous reports [10].

Results

Mutation of V2R gene

A 468G-A transition, resulting in a trp156-to-ter (W156X) substitution was identified in Family 1. In
Family 2, a 604C-T transition, resulting in an arg202-to-cys (R202C) substitution was found. A 839A-G transition, resulting in a tyr280-to-cys (Y280C) substitution were recognized in Family 3. A 320G-A transition, resulting in an gly107-to-glu (G107E) was identified in Family 4. All female carriers had both normal and abnormal alleles (Fig. 2).

**Assay for X-inactivation**

The results of GeneScan analysis of the androgen receptor CAG repeat are shown in Fig. 3. Peak height ratios for the two alleles in undigested samples and digested samples are shown in Table 3. The values of relative X-inactivation for normal allele were 38.1% in the mother of Family 1, 71.6% in the proband of Family 2, 79.4% in the mother of Family 3, 44.6% in the mother of Family 4, 91.2% in the grandmother of Family 4, and 39.3% in the aunt of Family 4.

**Discussion**

More than 150 kinds of mutations of the V2R gene have been identified to date. The mutations are scattered within the coding region and there is no hot spot. Of the mutations found in this study, only the W156X mutation identified in Family 1 is novel. Mechanism by which female carriers with heterozygote V2R gene mutations show NDI symptoms is explained by X-inactivation occurring preferentially to the normal allele of theV2R gene [5–7]. It has been reported that female carriers who show clinical symptoms similar to those of male patients have skewed X-inactivation in other X-linked recessive disorders, such as Duchenne muscular dystrophy [11], hemophilia B [12], Lesch-Nyhan syndrome [13], ornithine transcarbamylase (OTC) deficiency [14], X-linked myotubular myopathy [15], Fabry disease [16], sideroblastic anemia [17], and Lowe syndrome [18].
Fig. 3. GeneScan analysis of androgen receptor CAG repeat (a: Family 1, b: Family 2, c: Family 3, d: Family 4). In digested samples of female carriers, amplification occurs if the restriction sites are methylated, and amplification does not occur if the restriction sites are not methylated.
In our study, the normal X alleles of the asymptomatic mother of Family 1 and the asymptomatic aunt of Family 4 were inactivated 38.1% and 39.3% (random X-inactivation). Skewed X-inactivation occurring preferentially to normal X alleles was recognized in the proband of Family 2 (71.6%), the mother of Family 3 (79.4%), and the grandmother of Family 4 (91.2%) who showed clinical NDI symptoms. The results for these five female carriers suggest that the dominant methylation occurring to the normal allele of the V2R gene causes clinical NDI symptoms of the female carriers as previously reported. However, the degree of skewed X-inactivation in symptomatic female carriers is different between previous reports and our study. In previous reports [5–7], extremely or highly skewed X-inactivation was recognized in four out of five symptomatic female carriers (94.3%, 93%, 89%, and 84%). On the other hand, two out of three symptomatic female carriers had moderately skewed X-inactivation in our study. Although definitive proof can only be obtained by the analysis of renal tubular cells, moderately skewed X-inactivation may also cause clinical NDI symptoms of female carriers.

In Family 4, the grandmother, who showed severe clinical symptoms, had extremely skewed X-inactivation (91.2%). Although the grandmother was supposed to have a normal V2R gene on the same chromosome as the (CAG)$_{21}$ allele and a mutant V2R gene on the same chromosome as the (CAG)$_{11}$ allele, based on the result of GeneScan analysis, the mother had the (CAG)$_{14}$ and the (CAG)$_{21}$ alleles. This suggests that the mutant V2R gene of the grandmother moved to the (CAG)$_{14}$ allele from the (CAG)$_{21}$ allele via meiotic recombination.

In the mother of Family 4 who showed moderate NDI phenotype, the X-inactivation ratio observed for the normal X allele was 44.6% (random X-inactivation) in peripheral blood leukocytes. Moses et al. [3] reported a female carrier with heterozygote V2R gene mutation who showed half the normal factor VIII response to desmopressin as other asymptomatic female carriers, in spite of showing a total lack of antidiuretic response to desmopressin like that of male NDI patients. They speculated that the X-inactivation ratios were different between the tissues in this female carrier. Sharp et al. [19] reported that although there was a significant correlation of the X-inactivation ratios between each tissue in most individuals, the X-inactivation ratios within an individual varied widely between different cell lineages in some normal females. Therefore, we supposed that X-inactivation ratios may be different between peripheral blood leukocytes and renal tubular cells in the mother of Family 4. In other X-linked recessive disorders, differences between clinical phenotypes of female carriers and the X-inactivation ratios in peripheral blood were found. Sumita et al. [20] reported that no significant correlation was found between the X-inactivation ratio in blood and serum CK activity in Duchenne/Becker muscular dystrophy carriers. Orstavik et al. [21] reported that the wide range in plasma concentration of factor VIII and IX in haemophilia A and B carriers could not be explained by the X chromosome inactivation patterns in peripheral blood cells. Yorifuji et al. [14] reported that although the X-inactivation ratio of peripheral blood cells was not correlated with the OTC activity, the X-inactivation ratio of hepatic cells was well correlated with the OTC activity in female manifesting carriers with OTC deficiency.

Since it has been reported that the incidence of ex-

<table>
<thead>
<tr>
<th>Family</th>
<th>Female carrier</th>
<th>Peak height ratio for the two alleles</th>
<th>Relative X-inactivation ratio for normal allele (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Undigested sample</td>
<td>Digested sample</td>
</tr>
<tr>
<td>1</td>
<td>Mother</td>
<td>(CAG)$<em>{14}$/ (CAG)$</em>{16}$ = 1.15</td>
<td>1.88</td>
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<tr>
<td>2</td>
<td>Proband</td>
<td>(CAG)$<em>{14}$/ (CAG)$</em>{17}$ = 1.20</td>
<td>0.47</td>
</tr>
<tr>
<td>3</td>
<td>Mother</td>
<td>(CAG)$<em>{14}$/ (CAG)$</em>{18}$ = 1.22</td>
<td>4.73</td>
</tr>
<tr>
<td>4</td>
<td>Mother</td>
<td>(CAG)$<em>{14}$/ (CAG)$</em>{21}$ = 1.44</td>
<td>1.16</td>
</tr>
<tr>
<td>4</td>
<td>Grandmother</td>
<td>(CAG)$<em>{17}$/ (CAG)$</em>{21}$ = 2.46</td>
<td>0.24</td>
</tr>
<tr>
<td>4</td>
<td>Aunt</td>
<td>(CAG)$<em>{14}$/ (CAG)$</em>{14}$ = 1.16</td>
<td>1.81</td>
</tr>
</tbody>
</table>
tremely skewed X-inactivation (ratios 90 : 10–100 : 0) in peripheral blood is 9–16% in normal females [19, 22], the extremely skewed X-inactivation is considered not to be a rare phenomenon. Therefore, female carriers with heterozygote V2R gene mutations who show NDI symptoms might be more common than previously reported. We must observe the clinical symptoms of female carriers with heterozygote V2R gene mutations more carefully.

In conclusion, the clinical NDI phenotypes may correlate to the X-inactivation patterns in female carriers with heterozygote V2R gene mutations. However, in some female carriers we cannot predict the clinical phenotypes by the evaluation of the X-inactivation pattern in peripheral blood leukocytes, because X-inactivation ratios within an individual sometimes vary widely between different cell lineages.

References


