Detection of Skewed X-Inactivation in Two Female Carriers of Vasopressin Type 2 Receptor Gene Mutation

YOKO NOMURA, KAZUMICHI ONIGATA, TOMOHISA NAGASHIMA, SHIGENORI YUTANI, HIROSHI MOCHIZUKI, KANJI NAGASHIMA, and AKIHIRO MORIKAWA

Department of Pediatrics, Gunma University School of Medicine (Y.N., K.O., T.N., S.Y., K.N., A.M.), Maebashi, Gunma 371; and the Department of Metabolism and Endocrinology, Saitama Children’s Medical Center (H.M.), Iwatsuki, Saitama 339, Japan

ABSTRACT
Most cases of congenital nephrogenic diabetes insipidus (NDI) are inherited in an X-linked manner, which is due to the mutations of the vasopressin type 2 receptor (V2R) gene. However, recent reports have suggested female NDI patients with heterozygote V2R gene mutations. The mechanism of inheritance was thought to be skewed X-inactivation.

We present a family with congenital NDI. Three male members were diagnosed with NDI, and examination of their V2R gene revealed a G inserted at nucleotide 804 of the open reading frame. Three females in a NDI family were examined using methylation analysis of the polymorphic CAG repeat of the AR gene. All of them possess both the normal and abnormal genes.

The X-inactivation patterns of the female members were investigated via the detection of methylated trinucleotide repeat in the human androgen receptor gene. The grandmother showed extremely skewed methylation of one X chromosome, and the mother revealed moderately skewed methylation. The daughter of the grandmother’s sister, who has no symptoms of NDI, showed random methylation.

The highly skewed X-inactivation pattern of the grandmother suggests that her NDI phenotype is caused by dominant methylation of the normal allele of V2R gene. (J Clin Endocrinol Metab 82: 3434–3437, 1997)

CONGENITAL nephrogenic diabetes insipidus (NDI) is a hereditary disorder with defective renal responses to arginine vasopressin and is characterized by a failure to concentrate urine that results in polyuria and polydipsia (1).

Most cases of congenital NDI are transmitted in an X-linked recessive manner. A mutation in the vasopressin type 2 receptor (V2R) gene on the long arm of the X chromosome (Xq28) has been detected in an affected individual (2) and is thought to be the cause of the disease. Other modes of inheritance have also been described (3–6). Mutations in the aquaporin 2 (AQP2) gene have been found in families with autosomal recessive inheritance (7, 8). In cases of mutations in the V2R gene, female carriers are usually asymptomatic. However, in some cases, female carriers show a clinical phenotype similar to that of male NDI patients (9, 10).

Gene dosage compensation in humans is achieved through random inactivation of one of the two X chromosomes in the cells of normal females (11). When the X chromosome with a normal allele is inactivated preferentially, female carriers may be affected (12–15). Methylation-sensitive restriction enzymes may be used to verify the skewed X-inactivation. The HpaII and HhaI cleavage sites in the first exon of the human androgen receptor (AR) gene, which is one of the methylation sites in the inactive X chromosome, are highly polymorphic, and the maternal and paternal X chromosome activation status is detectable (16, 17).

Three females in a NDI family were examined in the present study. These women display different degrees of symptoms of NDI. Genetic analysis of the V2R gene showed that all three are heterozygous for a V2R mutation. In addition, the X-inactivation patterns of these three individuals were examined using methylation analysis of the polymorphic CAG repeat of the AR gene.

Subjects and Methods

NDI family (Fig. 1)
The male Japanese patient (III-4) was born at term after an uncomplicated pregnancy. His parents (II-4 and II-5) were nonconsanguineous. On the 55th day of life, he was admitted to hospital with vomiting and high fever and was cured by infusion with electrolyte solution. His mother (I-9) suffers from polyuria and polydipsia, but had never sought medical attention. I-1 died of unknown causes. I-5 died of a brain tumor. II-1, II-2, II-3, II-4, II-5, III-1, and III-3 present no NDI symptoms, and III-2 and III-5 suffer from polyuria, polydipsia, and hypernatremia.

The results of the water deprivation test and vasopressin loading test (Table 1) showed that the male patients (III-2, III-4, and III-5) and the grandmother (I-9) did not concentrate their urine osmolality even after the infusion of vasopressin. The mother (II-5) revealed an incomplete antidiuretic response to vasopressin loading. The activity of factor VIII coagulant was evaluated in the grandmother and the mother to reveal the extrarenal response of V2R. Factor VIII activity did not increase in the grandmother (108%) after vasopressin infusion, but increased moderately in the mother (280%).

Genetic analysis of V2R and AQP2
V2R and AQP2 were examined in patients I-9, II-1, II-2, II-5, III-2, III-4, and III-5. Genomic DNA was extracted from a peripheral blood sample from each patient using the phenol-chloroform method.

The samples were amplified using the PCR. Six sets of primers designed for the V2R gene were used and have been described previously (18). The PCR conditions were 30 s at 94 C, 30 s at 60 C, and 2 min at 72 C for 30 cycles. Each exon of the AQP2 gene was amplified through 30 cycles of 95 C for 1 min, 60 C for 1.5 min, and 72 C for 1.5 min, using...
TABLE 1. Water deprivation test and Pitressin loading test

<table>
<thead>
<tr>
<th>Urinary osmolality (mosmol/L)</th>
<th>Water deprivation</th>
<th>Pitressin loading</th>
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<tbody>
<tr>
<td></td>
<td>Vor</td>
<td>Peak</td>
</tr>
<tr>
<td>I-9</td>
<td>40</td>
<td>54</td>
</tr>
<tr>
<td>II-5</td>
<td>254</td>
<td>438</td>
</tr>
<tr>
<td>III-2</td>
<td>221</td>
<td>362</td>
</tr>
<tr>
<td>III-4 (proband) and I-9 (grandmother) showed no elevation of urinary osmolality, and II-5 (mother) revealed an incomplete response to pitressin.</td>
<td>125</td>
<td>251</td>
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Previously described primers (19). The existence of the mutation was screened by PCR-single strand conformation polymorphism (SSCP) using 5% Hydrolink-MDE gel (AT BioProducts, Malvern, PA) with 5% glycerol. The electrophoresis was performed at 200 V at 4 C for 6 h. Cycle sequencing reactions were performed using the TaqDyeDeoxy termination Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

Semiquantitation of X-inactivation

DNA samples from the carrier females (I-9, II-2, and II-5) were digested using methylation-sensitive restriction enzymes (10 U HpaII and 12 U HhaI) in a reaction volume of 25 μL at 37 C for 2 h. The PCR primers used for the AR locus, that included the HpaII and HhaI cleavage sites and the CAG repeat element, were previously described (16). The 5’-end of the forward primer was modified with fluorescein (6-FAM). Five microliters of digested and undigested samples were amplified for 30 cycles of 95 C for 45 s, 60 C for 30 s, and 72 C for 30 s.

When the DNA was initially digested, amplification only occurred if the restriction sites were methylated. The PCR products were electrophoresed with ROX mol wt markers for loading onto an ABI 310 automated sequencer (Applied Biosystems, Foster City, CA). Peak heights were analyzed using GeneScan software (Applied Biosystems). The major peak heights of two alleles were seen in the undigested PCR products, and to compensate for unequal amplification of alleles, a correction factor was defined by calculating the ratio between the two alleles. The peak heights of corresponding alleles in the digested products were measured, and the values were corrected using this factor. The sum of the two alleles was defined as 100% X-inactivation, and the degree of X-inactivation for each allele was expressed as a percentage of inactive X chromosomes.

Results

Mutation of V2R gene

PCR-SSCP of samples from three male (III-2, III-4, and III-5) and three female (I-9, II-2, and II-5) patients suggested the existence of a mutation in V2R gene (data not shown). The DNA sequence of these samples showed that a G had been inserted at nucleotide position 804, causing a frame shift that resulted in a premature stop codon at amino acid 258. The females had both normal and abnormal alleles (Fig. 1). The sequences of other exons of the V2R gene revealed no mutation.

No abnormalities of the AQP2 gene were observed in any individual using PCR-SSCP and direct sequencing.

Assay for X-inactivation

Peak height ratios for the two alleles in undigested samples were (CAG)20/(CAG)22 = 1.59 in I-9, (CAG)22/(CAG)24 = 1.03 in II-2, and (CAG)21/(CAG)23 = 0.86 in II-5 (Fig. 2). Correcting the results for digested samples yielded values of relative X-inactivation for one allele of 94.3% in I-9, 74.9% in II-2, and 65.8% in II-5.

Discussion

In this family, six individuals have a mutation (804insG) in the V2R gene, and all females examined have a normal allele in addition to the mutation allele. The grandmother, who displays the complete NDI phenotype with extrarenal involvement, showed extremely skewed X-inactivation.

The 804insG mutation, which causes a stop codon at amino acid 258 (3), results in no protein synthesis (20) and is thought to be responsible for the dysfunction of V2R.

Lieburg et al. described the clinical phenotype of NDI in female carriers with the V2R mutation (10). In three families, four females showed clinical features resembling the phenotype in male NDI patients. All affected females and two asymptomatic female members were shown to be heterozygous for the V2R mutation. Another female NDI case with marked vasopressin resistance was reported by Moses et al. (9). In that study, mother and daughter had both normal and abnormal alleles. The mother revealed a total lack of anti-diuretic response and half of the normal factor VIII response. The daughter had responses similar to asymptomatic female members of the family. The phenotypes of the mother and daughter may be due to...
mutation of the V2R gene (804insG) may present variable phenotypes. The skewed inactivation of the X chromosome may be an appropriate explanation for this variable expression.

In the present study, the X-inactivation status of DNA isolated from the peripheral blood of female carriers was examined using the polymorphic CAG repeat of AR locus. In the grandmother (I-9), who has the complete congenital NDI phenotype with extrarenal involvement, methylation of the X chromosome was extremely skewed. The mother, who had mild disturbance in the ability to concentrate urine in the water deprivation test, but no subjective symptoms, showed moderately skewed X-inactivation. The daughter of the grandmother’s sister displayed a normal increase in urine osmolality and random methylation of X chromosomes. These findings suggest that the degree of nonrandom X-inactivation correlated with the degree of expression of the NDI phenotype. As the V2R locus is on Xq28, and the AR locus is on Xcen-q13, crossing over most likely occurs between these loci (21). When translocation occurs, the V2R locus and the AR locus in the mother exist on different chromosomes. In fact, the AR trinucleotide repeat assay indicated that the proband has the (CAG)_{24} allele (data not shown), and that the grandmother has the (CAG)_{20} and (CAG)_{22} alleles. The results of GeneScan analysis suggested that the grandmother has the mutation allele of V2R, which is preferentially active, on the same chromosome as the (CAG)_{20} allele. These findings suggest that the mutation allele of V2R moved to a different allele via translocation. To obtain definitive proof that methylation in the normal allele of female NDI patient is dominant, the methylation of the locus adjacent to the V2R locus must be examined. However, other such suitable loci cannot be defined at present. Furthermore, extremely skewed X-inactivation in the X chromosomes of the grandmother indicates that NDI symptoms would be expressed in a female carrier when the normal allele is inactivated preferentially. Although the mechanism by which preferential methylation of the normal allele occurs is not clear, the possibility that the mutation affects the process of X-inactivation cannot be excluded. However, given that excessive lyonization (>90%) has been detected in 9% of healthy females (22), the skewed X-inactivation observed in this family may have occurred by chance.

In conclusion, the X-inactivation pattern of female carriers suggests that the variable NDI phenotype expressed by these carriers is caused by the degree of methylation of the normal V2R gene.

References