NOTE

Two Novel Aquaporin-2 Mutations in a Sporadic Japanese Patient with Autosomal Recessive Nephrogenic Diabetes Insipidus

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Abstract. We identified two novel mutations of the aquaporin-2 (AQP2) gene in a sporadic Japanese patient diagnosed with an autosomal recessive nephrogenic diabetes insipidus (NDI). The patient, a Japanese boy, was referred to our clinic at the age of 5 months because of unexplained recurrent fever. He was diagnosed with NDI by clinical, biochemical and endocrine findings. Molecular analysis demonstrated that he was a compound heterozygote for two mutations. One mutation consisted of a two base deletion in exon 1 (197, 198 delCA). This deletion caused a frameshift in the open reading frame, resulting in a premature stop codon 186 bases downstream in exon 1. The second mutation was a G to A transition of the terminal exon splice site (1502-1G→A). To date, several mutations in the AQP2 gene have been described, however no splicing mutation in the AQP2 gene has been identified. The deletion mutation described in this case study was inherited paternally and the splicing site mutation was inherited maternally, indicating an autosomal recessive inheritance. In the present case study, we identified two new mutations in the AQP-2 gene. Previous studies have shown that there is no hot spot for mutations in the AQP-2 gene, and thus genetic analysis for individual patients is helpful for genetic counseling and early diagnosis.

Key words: Nephrogenic diabetes insipidus (NDI), Aquaporin-2 (AQP2) gene, Splicing junction mutation, Deletion mutation

(RECENT molecular approaches have demonstrated that congenital nephrogenic diabetes insipidus (NDI) is caused by defects of at least two different genes, that is, the arginine vasopressin (AVP) receptor V2 gene in X-linked NDI and the vasopressin-sensitive water channel (aquaporin-2, AQP2) gene [1–3]. The gene for human AQP2 has been localized to chromosomal region 12q13 [2]. So far, several mutations in the AQP2 gene have been found in both the autosomal recessive and dominant forms of NDI. Since these mutations are distributed throughout the gene, any mutation hot spot is not present [4–11].

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Furthermore, in vitro studies of mutant AQP 2 proteins have clarified the mechanisms of loss of function and the differences of the inheritance [5, 6, 9–11].

Here, we report on a Japanese male patient with an autosomal recessive form of NDI. He was a compound heterozygote for a two base deletion mutation in exon 1 and a splicing acceptor site mutation in the terminal exon splice junction of the AQP2 gene.

Case Report

The male proband (Fig. 1A, II-2) was delivered to healthy Japanese parents following uncomplicated pregnancy. There was no family history of NDI, and his parents were not consanguineous. At the age of 5 months, he was referred to our hospital because of unexplained recurrent fever of 3 months duration. At
admission, his serum sodium and potassium levels were 152 mEq/L and 4.5 mEq/L, respectively. Serum osmolality was elevated (312 mOsm/kg), despite a low urinary osmolality (92 mOsm/kg). Plasma AVP measured by radioimmunoassay was extremely high (79.3 pg/ml). Nasal 1-desamino-8-D-arginine vasopressin (DDAVP) administration did not increase urine osmolality. Brain magnetic resonance imaging (MRI) showed normal anterior pituitary gland and pituitary stalk, but high intensity in the posterior lobe disappeared, suggesting increased vasopressin release. Blood urea nitrogen increased slightly (25 mg/dl) due to dehydration and serum creatinine was normal (0.4 mg/dl). Urinary excretion of β2-microglobulin was not found. An abdominal computed tomography scan and ultrasonographic examination showed that both kidneys were normal. Based on these findings, he was diagnosed as having NDI. He was treated with intravenous fluid administration, and subsequent hydrochlorothiazide (1 mg/kg), and has been well controlled.

**Polymerase-chain-reaction (PCR) and direct sequencing of the AQP2 gene**

Institutional review board approval of the research protocol and informed consent from parents were obtained. Genomic DNA was extracted from leukocytes according to standard procedure. We selected primers amplifying the V2 receptor and AQP2 gene, and PCR was performed according to previous publications [2, 12]. PCR products were purified on a 1.0% agarose gel and were sequenced by automated DNA sequencing employing Taq DyeDeoxy sequencing reagents (Applied Biosystems, Forester, CA).
Results

At first we determined the nucleotide sequence of the V2 receptor, but this gene was found to be completely normal. Analysis of the AQP2 gene showed two mutations (Fig. 1A). One was a two base deletion (197, 198 delCA) at codon 43 in exon 1 (Fig. 1B). This frameshift mutation alters the open reading frame (ORF), resulting in a premature stop codon 186 bases downstream in exon 1. The other mutation was A to G transition in intron 3 of the terminal exon splicing junction (1502-1 G→A), and this mutation occurred at the conserved nucleotide of a splice acceptor site (Fig. 1C). A study of the parents’ relevant gene sequences revealed that the deletion mutation was transmitted paternally, and the splicing mutation was transmitted maternally, confirming an autosomal recessive inheritance (Fig. 1D).

Discussion

We have reported of a patient with autosomal recessive NDI caused by mutations in the AQP-2 gene. He showed recurrent unexplained fever and mild growth failure. All these findings are consistent with features of NDI. The posterior pituitary bright spot of T1-weighted brain MRI was absent. This can be explained by increased vasopressin release in NDI [13]. Molecular analysis showed two novel mutations in the AQP2 gene (197, 198 delCA and 1502-1 G→A) and an autosomal recessive inheritance in this family. Since 197, 198 delCA mutation introduces a premature stop codon in exon 1, this mutation might produce a truncated protein, resulting in impaired function in the AQP channel. One nonsense mutation of exon 1 (G100X) in a large cluster of patients with autosomal recessive NDI has been reported [7]. In addition, Lin et al. have demonstrated that two missense mutations (Q57P and G100V) in exon 1 cause the loss of function of mutant AQP2 proteins in vitro [11]. These findings suggest that the truncated protein cannot exert its proper water channel function. The other mutation, 1502-1G→A, exists in a terminal exon splice junction. Generally, splicing errors are well-recognized causes of human genetic disorders [14], however to date any splicing mutations in the AQP2 gene have not been reported. Otterness et al. reported a mutation in the terminal intron of the thiopurine methyltransferase (TMPT) gene in human thiopurine intolerance. According to their study, mRNA from the allele bearing the splicing junction in the terminal exon was not identified by reverse-transcriptase PCR due to an aberrant mRNA splicing or mRNA instability [15]. Similar to this mechanism, no transcript can be produced in our case. The other possibility is that alternative splice—acceptor sites downstream from the mutation site may be used. Any protein translated from this mutated gene would be different form those of the wild-type protein, because the sequence coding exon 4 in the mutant gene results in a defective amino acid sequence and/or different protein. Several in vitro studies of mutant AQP2 proteins identified in autosomal dominant NDI patients indicate the importance of the C-terminal domain encoded by exon 4 for the intracellular trafficking of AQP2 [9, 10]. Thus, it is difficult to imagine such a mutant derived from any alternative splice site as being functional. Further in vitro analysis of the mutations described in this study will clarify the molecular mechanism of NDI.

To date several mutations in the AQP2 gene have been described, and scattered throughout all exons of the AQP2 gene. Therefore, direct analysis of mutations in the AQP2 gene will be required for each individual with NDI. This will be of help to the accurate diagnosis of patients, early initiation of appropriate therapy and genetic counseling of carriers of the disease.

References


