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MOLECULAR ANALYSIS OF AVPR2 GENE IN A JAPANESE PATIENT WITH X-LINKED NEPHROGENIC DIABETES INSIPIDUS, CONGENITAL HEART DISEASE AND MENTAL RETARDATION

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X-linked nephrogenic diabetes insipidus (NDI) is a rare disease with defective responses of renal and extrarenal arginine-vasopressin V2 receptors (AVPR2). This is caused by mutations in the AVPR2 gene located in chromosome region Xq28. We experienced a 35-year-old male patient with NDI accompanied by severe congenital heart disease (CHD), double-outlet right ventricle (DORV), ventricular septal defect and pulmonary hypertension, as well as mental retardation. DORV is a rare CHD, and patients with such a CHD often die during childhood without surgical treatment. There have been no reports of NDI accompanied with such a severe CHD. We observed clinically and performed a genetic analysis on this patient. A missense mutation (R202C) in the AVPR2 gene from the patient was identified. One of the reasons why this patient has been able to survive so long without surgical intervention may be the reduced cardiac preload due to polyuria of NDI as well as diuretics used.

Key words: nephrogenic diabetes insipidus, vasopressin, double-outlet right ventricle, mutation.

INTRODUCTION

Congenital nephrogenic diabetes insipidus (NDI) is characterized by an inability to concentrate the urine despite normal or elevated levels of blood antidiuretic hormone (ADH) (1). It is attributed to a defect in one of two different genes: one is the arginine-vasopressin (AVP) V2 receptor (AVPR2) gene which is mapped to Xq28, and the other is the aquaporin 2 receptor (AQP2) gene, mapped on 12q13. Both AVPR2 and AQP2 have antidiuretic action in the renal collecting ducts. About 90% of patients are males with X-linked recessive NDI. An estimate of the incidence is about 1 in 152,000.

Affected individuals present polyuria, fever, vomiting, and constipation due to hypertonic dehydration from early infancy. Mental retardation and physical growth failure are the classical "historical" consequences of a late diagnosis or lack of treatment (2). The gene sequence encoding the human AVPR2 was isolated and characterized (3-5). More than 100 types mutations have been identified so far (6). We analyzed the AVPR2 gene in a Japanese male with X-linked NDI accompanying mental retardation and congenital heart disease (CHD) including double-outlet right ventricle (DORV), ventricular septal defect (VSD) and pulmonary hypertension (PH). We describe here the AVPR2 gene mutation as well as clinical and biochemical findings of this patient.

CASE PRESENTATION

A 35-year-old male born to unrelated parents was investigated. His birth weight was 2,500 g, and cyanosis and heart murmur were noticed soon after birth. DORV, VSD and PH were diagnosed at 2 months of age. He had polydipsia and polyuria from childhood, and mental retardation was also noted since 3 years of age. According to his previous clinical record, he had been treated with thiazide for NDI as well as digoxin for the CHD. He visited our hospital at around 12 years of age, when he had already Eisenmenger syndrome. Since then, only trichloromethiazide has been given for control of NDI,

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without any cardiotonic drugs. Since his adolescence, the daily volume of urine was around 5 liters, and specific gravities were consistently around 1.005. In a urine test at the age of 30 years, the osmolality (UO) was 141 mOsm/l; the concentration of serum antidiuretic hormone (ADH) was 12 pg/ml (normal range, 0.3-3.5 pg/ml). No significant increase of UO was observed in response to a Pitressin® test (the maximum was 173 mOsm/l). Both his parents had severe mental retardation. The mother died 10 years ago at the age of 68 years, and the father is now 80 years old.

Delay in physical growth was noted since his childhood, and now his height is 158 cm and his weight is 50 kg. Now, he has been receiving allopurinol (300 mg/day) and trichloromethiazide (2 mg/day) since 12 years old. Abdominal CT examination performed recently revealed moderate pyelectasia and ureterectasis in the right side, as shown in Fig. 1.

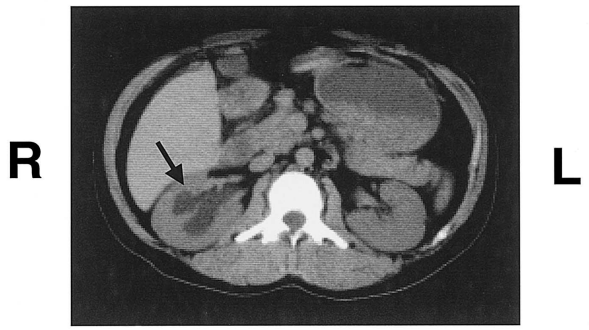

Fig. 1. Abdominal CT. Moderate pyelectasia is seen at the right kidney as indicated with an arrow. The left kidney was in a normal range.

MATERALS AND METHODS

Analysis of AVPR2 gene: cDNA was amplified from total RNA by reverse transcriptase-polymerase chain reaction (RT-PCR). Sequences of primers for PCR (Table 1) were designed as shown in Figure 2.

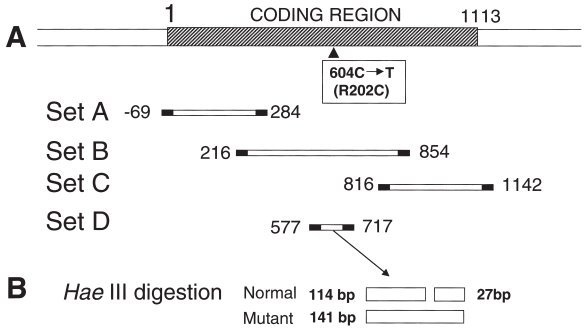

Fig. 2. Design of AVPR2 gene DNA analysis.
A: Coding region of AVPR2 gene and the primer sets designed. Coding region (1 to 1113) is indicated with hatched bar; a mutation point is indicated with an arrowhead. DNA fragments used for restriction-enzyme assay are shown in the boxes; primers used for PCR are shown with black boxes at the both ends of respective DNA fragments.
B: *Hae* III digestion of DNA fragment coding 577 to 717. DNA fragments of normal control (wild-type) were digested to two bands, 114 bp and 27 bp, while that of the patient was not.

PCR was run with 0.5 μg of cDNA, 12.5 mM of primers, 10 μM each of dNTPs, 5 μl of 10×buffer with $MgCl_2$, and 1.25 U of Taq polymerase in an aliquot with total volume of 50 μl. PCR condition was as follows: 94°C for 60 sec for denaturation, 60°C for 60 sec for annealing, and 72°C for 60 sec for polymerization at 30 cycles. The amplified products were electrophoresed on 2% agarose gel, and then the expected band was cut to extract the DNA fragments, using a QIAquick Gel Extraction Kits (QIAGEN GmbH, Hilden, Germany).

Direct sequence of AVPR2 gene: Direct sequencing of purified PCR products was carried out for 25 cycles with incubation at 96°C for 60 sec for denaturation, at 50°C for 15 sec for annealing, and then at 60°C for 4 min for extension. Then samples were purified according to procedures with Thermo Sequence™ dye terminator cycle sequencing pre-mix

Table 1. Sequence of primers used for direct sequencing or restriction enzyme assay.

	Sense primer (5'-3')	Antisense primer (5'-3')
set A	(-69)-tgctcctcaggcagaggctga-(-49)	284-ggcagcacttggaacagag-266
set B	216-acccatacacgtcttcattggc-237	854-gcccagcacacacatagacgacca-829
set C	816-gacgctagtgattgtggtcgt-836	1142-ctagaggcaagacacccaacagctc-1118
set D	577-tgggcctgctttgcggagccctgg-600	717-ccctggcaccagactggcat-698

kit (Amersham, Cleveland, OH, USA), and the sequence was carried out with an ABI 310 gene analyzer (PE Applied Biosystems, Foster, CA, USA).

Restriction enzyme digestion analysis: Restriction enzyme, *Hae* III was purchased from TaKaRa SHUZO CO., LTD (Tokyo, Japan). Nested PCR was carried out using the primer set D (Figure 2) to amplify the 141-bp fragment, which carried the mutation. Wild-type DNA fragments had a site for *Hae* III, while the mutant did not. The DNA fragment was incubated with 10 units of *Hae* III enzyme and then subjected to electrophoresis on an 8% polyacrylamide-TAE (Tris/Acetate/EDTA) gel.

RESULTS

Sequence of AVPR2 gene: Direct sequencing revealed the C to T transition at nucleotide 604 (604C to T), which caused amino acid substitution Arg to Cys at codon 202 (R202C), as shown in Fig. 3.

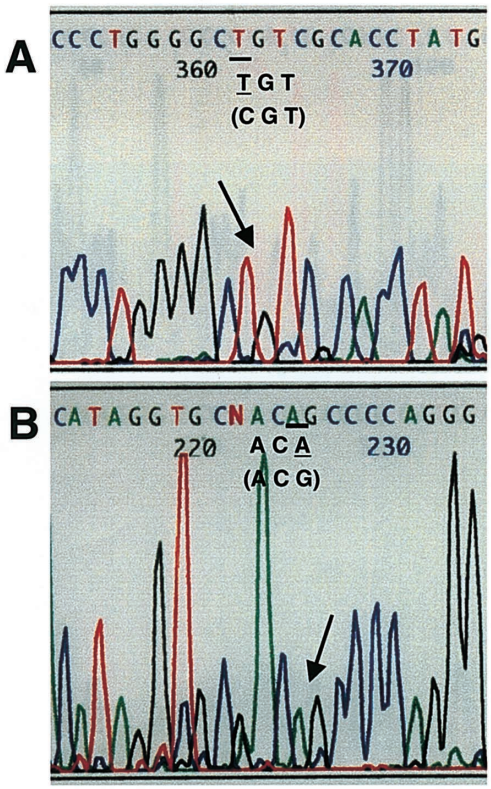

Fig. 3. Direct sequence of PCR products.
A: Direct sequence of PCR products using the sense primer of set B. CGT (Arg) was substituted to TGT (Cys).
B: Direct sequence of the same PCR product using the reverse primer of set B. GCA was changed to ACA. Peak of nucleotide change is indicated with an arrow.

Restriction enzyme digestion assay: *Hae* III restriction enzyme assay revealed that the DNA fragment of 141-bp from control was digested into two bands, 114-bp and 27-bp, while the 604C to T mutant was visible as a 141-bp band (Fig. 4). The existence of 604C to T mutation in the AVPR2 gene of our patient was confirmed. Family analysis of the mutation was not available.

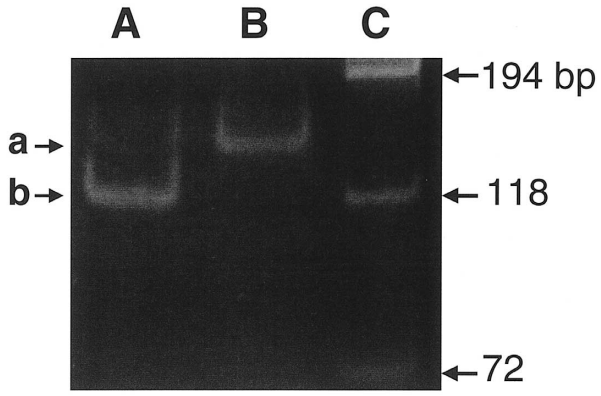

Fig. 4. Restriction-enzyme digestion assay with *Hae* III.
A: Control, B: Patient, C: DNA size marker. Bands 'a' and 'b' indicate 141 bp and 114bp, respectively. Bands of 194 bp, 118 bp and 72 bp are DNA molecular size markers used.

DISCUSSION

More than 100 types of mutations in AVPR2 gene that cause NDI have been reported up to now (6). As illustrated in Fig. 5, fifteen mutant genes, including R202C, have been identified among Japanese pa-

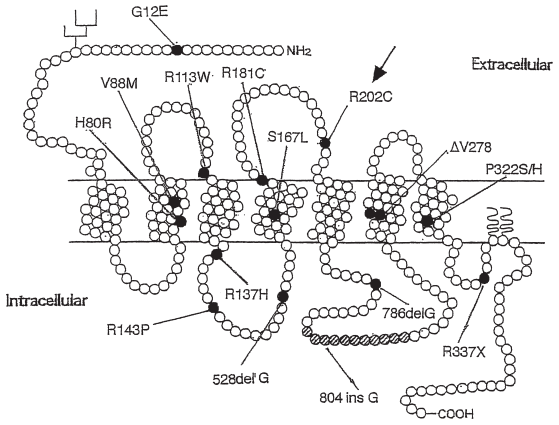

Fig. 5 AVPR2 mutations found among Japanese patients. Missense mutations are: G12E, H80R, V88M, R113W, R137H, R143P, S167L, R181C and P322S/H. Nonsense mutations are: 528delG, 786delG, 804insG, R337X and ΔV278. The R202C mutation found in our patient is indicated with an arrow.

tients: G12E, H80R, V88M, R113W, R137H, R143P, S167L, R181C, P322S/H, 528delG, 786delG, 804insG, R337X and ΔV278 (7-13). Previously, the mutation of R202C was reported as a recurrent mutation (2), which arose *de novo* in unrelated families and ethnic groups, including Italian, Netherlander, American, and Japanese, as well. It was reported that AVPR2 mutants could be classified into at least three distinct phenotypes: (a) mutants with lost binding activity, (b) transport-defective mutants, and (c) biosynthesis-defective mutants (14). Expression of R202C mutant performed by Tsukaguchi *et al* (7) revealed that R202C mutation was responsible for NDI due to simple binding impairment at the cell surface.

Bichet *et al* reported that no significant correlation between phenotypic expression and genotype was found in NDI patients in their study (1). However, Lieburg *et al* (15) reported that only a mutation, G185C, resulted in a mild phenotype. The G185C mutation is located in the second extracellular loop of the AVPR2, which is the probable AVP binding region. The mutation of R202C found in our patient was also located in this extracellular loop, and our patient showed a relatively mild clinical phenotype of NDI.

This case had not only NDI but also CHD including DORV with VSD and PH. There have been no reports of NDI with CHD. Patients with such a severe CHD like this case may die during childhood without surgical intervention because of PH or cardiac failure (CF) (16). We supposed that the absence of severe PH or CF in this patient was a result of the reduced cardiac preload due to NDI and the diuretics used in childhood. It is likely a reason why this patient has been able to survive so long without CF or other complications.

Recently a positive inotropic effect of vasopressin at low doses was observed under special conditions such as Langendorff heart preparation or overexpression of V2R in the heart (17, 18). On the contrary, it was reported that vasopressin decreases cardiac output by constricting coronary arteries in normal conditions or in mild experimental CF (19, 20). Since no evidence to suggest coronary ischemia was found in our patient, it was interpretated that the concentration of vasopressin was not so high to develop coronary ischemia. Hence we did not exclude a possibility of positive inotropic effect of vasopressin in our case.

NDI patients often have mental retardation, probably due to repeated episodes of hypertonic dehydration without appropriate treatments. In this case, however, there is also a possibility that his mental retardation was due to other genetic reasons, because both of his parents also had severe mental retardation.

The patient has received trichloromethiazide for NDI for at least 20 years. Urologic complications as a consequence of polyuria in patients with NDI were reported various from mild unilateral dilation of the urinary tract to severe bilateral hydronephrosis (15, 21). The abdominal CT scan of this case showed a moderate pyelectasia and ureterectasis in the right kidney. There may be a possibility that the unilateral enlargement was caused by a minor abnormality at the ureterovesical junction, which escaped detection by abdominal CT, as well as caused by polyuria of NDI.

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