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A Novel Mutation in the Arginine Vasopressin Receptor 2 Gene Causing Congenital Nephrogenic Diabetes Insipidus

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What is already known on this topic?

About 90 percent of all cases of hereditary nephrogenic diabetes insipidus result from mutations in the *AVPR2* gene. To date, more than 250 mutations have been identified comprising missense, nonsense, small insertions and deletions, large deletions and complex rearrangements in *AVPR2* gene.

What this study adds?

In this study, we found a novel hemizygous missense mutation in *AVPR2* gene at the position 80th in exon 2 (p.H80Y) causing CNDI in a 6-year-old boy presenting with growth failure and dull normal cognitive functions.

Abstract

Objective: Congenital nephrogenic diabetes insipidus (CNDI) is a rare inherited disorder characterized by a renal insensitivity to the arginine vasopressin (AVP).

In the majority of the cases, CNDI is caused by the mutations in the *AVPR2* gene. Our objective is to report a novel mutation in *AVPR2* gene causing CNDI in a 6-year-old boy presenting with growth failure and dull normal cognitive functions.

Methods: The proband was the third off-spring of non-consanguineous parents and had polyuria (4.3 L/day), polydipsia (5 L/day). The diagnosis of CNDI was established by a water-deprivation and desmopressin challenge test. The genetic studies were also carried out in mother, siblings and affected family members due to they also had excessive fluid intake and diuresis. All exons of *AVPR2* gene for all participants were amplified and sequenced. Bioinformatics analysis for wild-type and mutant *AVPR2* were obtained with Swiss-Model and UCSF Chimera 1.10.2.

Results: A novel hemizygous missense mutation was identified at the position 80th in exon 2 (p.H80Y) of *AVPR2* in the proband. The proband's mother, maternal aunt and grandmother were heterozygous and maternal uncle was hemizygous for this mutation. According to bioinformatic analysis, this mutation leads to significant conformational changes in protein structure.

Conclusion: p.H80Y mutation can obviously cause inappropriate folding of the protein. Therefore, the pathway of water homeostasis via *AVPR2* and AVP can be improper and it can be the reason of DI. We suggest that future functional investigations of the H80Y mutation may provide a basis for understanding the pathophysiology of the NDI.

Keywords: *AVPR2*, Congenital nephrogenic diabetes insipidus, Mutation

Introduction

Water is vital, and hydration is important for physical and mental performance. Water balance of the body is controlled through fluid intake, which is stimulated by thirst and renal excretion of water as urine (1, 2, 3).

Increase in plasma osmolality or decrease in blood volume leads to secretion of the arginine vasopressin (AVP), also called as antidiuretic hormone (ADH), from the posterior pituitary gland (4, 5). AVP increases water permeability of renal collecting ducts by activating Arginine Vasopressin Receptor 2 (*AVPR2*), a type of G protein-coupled receptor, located on the basolateral membrane of the kidney collecting duct cells (6, 7). Binding of AVP to *AVPR2* activates

Gs/adenylate cyclase and leads to a series of intracellular events resulting in exocytic insertion of the water channel aquaporin-2 (AQP2) from intracellular storage compartments into the luminal membrane. This results in water reabsorption from the pro-urine by kidney tubule after an osmotic gradient (1, 8, 9). Any impairment in this pathway can lead to a metabolic disease called diabetes insipidus (DI) (10). DI has two major types; a deficiency of AVP causes central diabetes insipidus (CDI), whereas inadequate response of the kidney leads to nephrogenic diabetes insipidus (NDI) (11, 12).

Congenital NDI (CNDI) is an inherited form of NDI and this disorder is caused as a result of **loss-of-function** mutations of *AVPR2* gene or *AQP2* gene. *AQP2* gene defects cause to autosomal recessive and dominant NDI and it is responsible for a small percentage of the disease. However, **loss-of-function** mutations in *AVPR2* lead to X-linked recessive NDI and this accounts for 90% of cases with CNDI. In addition, X-linked NDI occurs with a frequency of 4-8/1 million male live births (13, 14, 15, 16). The well-known clinical symptoms of congenital NDI are polydipsia, polyuria, hypernatremia and hyperchloraemia (17, 18). **The reason for these symptoms is that the kidney loses its ability to concentrate urine caused by loss-of-function mutations of *AVPR2* (19).**

In this study, we described a novel hemizygous missense mutation causing a conversion of the histidine residue to tyrosine in the protein sequence, at the position 80th in exon 2 in a 6-year-old proband with symptoms of CNDI. X-linked recessive family pedigree was created by working in affected and unaffected family members.

Material and methods

Case Presentation

A 6-year-old boy was referred to the Pediatric Nephrology Department of Kecioren Research and Training Hospital for abnormal fluid intake (5 L/day) and diuresis (4.3 L/day) which was reported to have started in the early phases of his life. The parents indicated that it was possible to make him stop crying only if he drinks water in addition to breast milk. His height and weight were below of his peers during childhood and he succeeded moderately well in the preschool activities when compared to his peers. He was the third off-spring of non-consanguineous parents. His maternal grandmother, aunt and uncle were also reported to have excessive fluid intake and diuresis. It was his first admission to a medical center for the symptoms of polyuria and polydipsia. On physical examination, his height (104 cm, SDS: -2.66) and weight (14 kg, SDS: -3.92) were below the 3rd percentile, his blood pressure and skin turgor were normal. A laboratory examination on admission disclosed the following values: serum sodium at 137 mmol/L (range: 136-146), potassium at 4.86 mmol/L (range: 3.5-5.1), chloride at 102 mmol/L (range: 101-109), calcium at 9.52 mg/dL (range: 8.8-10.6), phosphorus at 5.67 mg/dL (range: 4-7), albumine at 4.07 g/dL, urea at 23 mg/dL and creatinine at 0.59 mg/dL. Urine specific gravity was 1.000. He had high calculated serum osmolality [281.5 mOsm/kg using the following formula: $2x[Na] + (BUN \div 2.8) + (glucose \div 2.8)$], low calculated urinary osmolality [67.7 mOsm/kg using the following formula: $1.86x[Na] + (BUN \div 2.8) + (glucose \div 2.8) + 9$] and hyponatriuria (15 mmol/L) on admission. The water deprivation test was stopped due to weight loss higher than %3 at 3.5th hour of the test. The plasma vasopressin (16.75 pmol/l, range: 0-13) drawn at the conclusion of the dehydration test was high, and the urine

parameters showed insignificant changes. **A desmopressin challenge test was performed. The administration of desmopressin 20 µg intranasally did not have any effect on urine parameters, either (Table 1).** There were continuously high urine output, low urine osmolality and impaired ability to increase urine osmolality to normal levels after ADH administration. He had stable blood pressure (112/75 mmHg) and heart rate (82/min) during the test. His urinary ultrasonography and pituitary magnetic resonance imaging showed no abnormality. On the verbal and performance tests of the Wechsler Intelligence Scale for Children (WISC -IV), the child obtained a score of 81 and 82, respectively. Both verbal and performance tests indicated a “dull normal” intellectual functioning. The patient was treated with thiazide diuretics (2 mg/kg/day), indomethazine (2 mg/kg/day), low-protein diet, low sodium diet and unlimited amounts of fluid after when he thrived. The patient responded well to the treatment during the first year; fluid intake (3-3.5 L/day) and diuresis (2.7-3 L/day) were both diminished. The patient never developed hydronephrosis nor experienced dehydration and/or hypernatremia during follow-up. The family pedigree was compatible with presumably X-linked recessive CNDI due to similar symptoms associated with NDI in some of family members (Figure 1).

The collection of blood samples from the proband and his family members were approved by the Ethics Committee of the Faculty of Medicine of Hacettepe University (approval no: 2607) and a written informed consent was obtained from all participants and their parents.

DNA isolation

Genomic DNA was extracted from peripheral blood leukocytes following a standard protocol using QIAamp[®] DNA Blood Mini Kit (QIAGEN, Germany). The extracted genomic DNA was quantified spectrophotometrically (QUAWELL) and stored in aliquots at -20°C until use.

PCR amplification and direct sequencing of AVPR2 gene

The entire coding regions and flanking intronic sequences of the *AVPR2* gene were amplified from genomic DNA using polymerase chain reaction (PCR). The sequences of the primers that were used in PCR are as follows: Exon 1, forward 5'-GTC TGACCA TCC CTC TCA ATC TTC-3' and reverse 5'-GGA GTC GGG AAG AGG GCC TGG TTA-3'; Exon 2a, forward 5'-ATA ACA TGG CTT CCT GGA GTC CC-3' and reverse 5'-TGC GCT GGG CGA AGA TGA AGA GCTG-3'; Exon 2b, forward 5'-TGG AAG TGG GGC TCACTG GAA CCG GC-3' and reverse 5'-GCT GTT GAG GCT GGC CAG CAA ACA TG-3'; Exon 3, forward 5'-TGT AGC CGT GGC TAG GGC TGA CGG G-3' and reverse 5' CCT GCC CCA GGA AGG CAG CTG AGC-3'. All PCR amplifications were performed under following conditions: 45 s 95 ° C, 45 s 64 ° C and 45 s 72° C for 32 cycles. After amplification, PCR products were separated by electrophoresis in 1.5% agarose gel. These products were purified enzymatically and sequenced using the Big Dye kit (Applied Biosystems, USA).

Analyses of bioinformatics

Three-dimensional protein structures for wild-type and mutant AVPR2 proteins, comparing amino acid sequence properties and predictions of binding sites of these proteins were obtained with computational tools such as Swiss-Model and UCSF Chimera 1.10.2 servers.

To predict the functional effect of mutation on the AVPR2, it was also analysed using PolyPhen-2 (Phenotyping Polymorphism v2) software.

Results

All of the coding regions for the *AVPR2* gene of the proband was screened by DNA sequence analysis. DNA sequence analysis revealed the presence of a novel hemizygous missense mutation at coding position 238 (c.C238T) (Figure 2). This mutation lead to a conversion of the histidine residue to tyrosine in the protein sequence, at the position 80th in exon 2. After the screening of the novel mutation, we also performed sequence analysis to proband's close family members. According to the results, his mother (II-6), maternal aunt (II-5) and grandmother (I-2) were heterozygous and maternal uncle (II-3) was hemizygous for this mutation (Figure 2). No mutation in *AVPR2* gene is detected in other family members (II-2, II-4, III-1, III-2, III-3).

According to bioinformatic analysis based on DNA sequence, we made a prediction on mRNA structures of the wild-type and mutated proteins. H80Y mutation is located in the second transmembrane domain (positions 78-98) of the protein (20). Hypothetically, we found some differences between alpha-helix and beta-sheet structures of wild-type and mutant AVPR2 proteins by the Swiss-Model and UCSF Chimera 1.10.2 servers (Figure 3). Comparisons of amino acid sequences revealed that theoretical pI values of wild type and mutant protein are 9.49 and 9.47, respectively and the theoretical molecular weights are 40,279.09 and 40,305.12, respectively. In addition, PolyPhen analyses predicted the mutation's effect with a score of 0.999 (out of 1), sensitivity of 0.14 and specificity of 0.99, therefore pH80Y mutation was identified as a probable damaging mutation.

Discussion

Congenital NDI is a rare disease, most commonly, caused by mutations in the AVPR2 in collecting duct epithelial cells which is encoded by *AVPR2* gene (Xq28). The gene encodes a 371-amino acid G protein-coupled receptor (GPCRs) with seven transmembrane, four extracellular and four cytoplasmic domains (7, 14). *AVPR2* mutations causing CNDI can vary in their functional severity; clinical symptoms and the response to DDAVP can be diverse.

CNDI is a severe form of DI and is difficult to treat. It is commonly due to inherited defects in children (14). The urine output in patients with CNDI can be lowered with a low-salt, low-protein diet, thiazide diuretics and/or potassium-sparing diuretic (amiloride), and nonsteroidal anti-inflammatory drugs (NSAIDs) (21). Diuretics in NDI patients reduce the urine output by promoting the reabsorption of sodium and water in the proximal tubule, thus delivering less

water to the collecting ducts (22). The inhibitory effect of indomethacin on urine volume is thought to be mediated by an AVP independent water reabsorption resulting from an increase in solute reabsorption and consequent medullary hypertonicity (23). Nevertheless, many patients still experience significant polyuria and polydipsia while receiving these therapeutic measures. The investigational therapeutic strategies for congenital NDI include the rescue of AVPR2 mutants by chemical chaperones and by passing defective AVPR2 signaling (24). In infants, early recognition is very important as the proper treatment can avert the physical and mental retardation that results from repeated episodes of dehydration and hypernatremia. Patients with congenital NDI should be monitored for growth, serum sodium concentration and hydration status, and development of hydronephrosis. Genetic analyses for CNDI can also be very useful for diagnose at an early age and should be performed in all patients with a family history of the disorder (14, 21, 25). Therefore, the definitive diagnosis of CNDI will provide appropriate genetic counseling and development of specific treatment strategies.

More than 250 putative disease-causing *AVPR2* mutations have been found comprising missense, nonsense, small insertions and deletions, large deletions and complex rearrangements in *AVPR2* gene to date (14, 26). The most common category of *AVPR2* mutations causing NDI are missense mutations. Many disease-causing mutations occur in the transmembrane domains compared to extracellular or intracellular domains. The *AVPR2* missense mutations are likely to impair folding and lead to rapid degradation of the misfolded polypeptide (14, 25, 26, 27).

We report here a male child with repeated episodes of dehydration, polyuria, and polydipsia in early infancy. The water deprivation test and the desmopressin challenge test confirmed the diagnosis of CNDI. The genetic analysis revealed a novel X-linked recessive missense mutation (p.H80Y) causing shift of histidine residue to tyrosine in the protein sequence of *AVPR2*. The H80Y mutation presented in this study, changed histidine, a basic, polar, positively charged amino acid, to tyrosine, an aromatic, non-polar amino acid. The function of altered AVPR2 structure is presumably significantly impaired as tyrosine join in beta-strand conformations in proteins (26). In addition, histidine and tyrosine amino acids have different physicochemical properties. Therewith, an altered protein conformation might impair the intracellular trafficking of AVPR2 and affect proper localization of the receptor into plasma membrane or decrease the AVP binding characteristics (20). The novel mutation reported in this study is located in the second transmembrane domain of the protein. This mutation point (codon 80) is a conserved residue among rat V1 and V2 vasopressin receptors and the human oxytocin receptor (28, 29). Another missense mutation at codon 80 (p.H80R) was also reported by Yuasa and his colleagues and they emphasized the sequence conservation and functional

importance of this codon (20). Our study describes a novel missense mutation (p.H80Y) in this same codon.

The functional significance of this mutation was analysed by utilizing the PolpPhen-2 software. This software can be used to predict the consequence of an amino acid change on the structure and function of a protein using physical and evolutionary comparative considerations (18, 30). As a result of the analysis, the H80Y mutation was identified as a probable pathogenic mutation. In addition, according to the bioinformatic analysis of this mutation, protein conformation is predicted to be impaired which can lead to an abnormal protein function. However, the functional analyses of this mutation is needed to determine the structure-function relationship in patients with CNDI. In conclusion, this study reports the clinical and molecular characterization of congenital nephrogenic diabetes insipidus and emphasizes the importance of the definitive diagnosis of CNDI.

Declaration of interest

This study is not subject to any conflicts of interest.

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Ethics

The collection of blood samples from the proband and his family members were approved by the Ethics Committee of the Faculty of Medicine of Hacettepe University (approval no: 2607) and written informed consent was obtained from all participants.

Authorship Contributions

Concept: Hatice Mergen, Asli Celebi Tayfur Design: Hatice Mergen, Asli Celebi Tayfur, Data Collection or Processing: Asli Celebi Tayfur, Tugce Karaduman, Merve Ozcan Turkmen, Analysis or Interpretation: Tugce Karaduman, Merve Ozcan Turkmen, Dilara Sahin, Literature Search: Tugce Karaduman, Merve Ozcan Turkmen, Dilara Sahin, Writing: Asli Celebi Tayfur, Tugce Karaduman, Merve Ozcan Turkmen.

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Table 1. The water-deprivation test and desmopressin challenge test results of the patient

	Weight (kg)	Urine density	Urine sodium (mmol/L)	Urine osmolarity (mOsm/kg)	Serum sodium (mmol/L)	Serum osmolarity (mOsm/kg)
Before the tests	14	1.000	15	67.7	137	281.5
1st hour	13,9	1.005	13	63,5	ND	ND
2nd hour	13.7	1.005	12	60,5	138	285.3
3.5th hour- desmopressin given	13.5	1.005	13	70,1	141	291.4
5th hour	ND	1.005	13	65.9	ND	ND

ND: not done

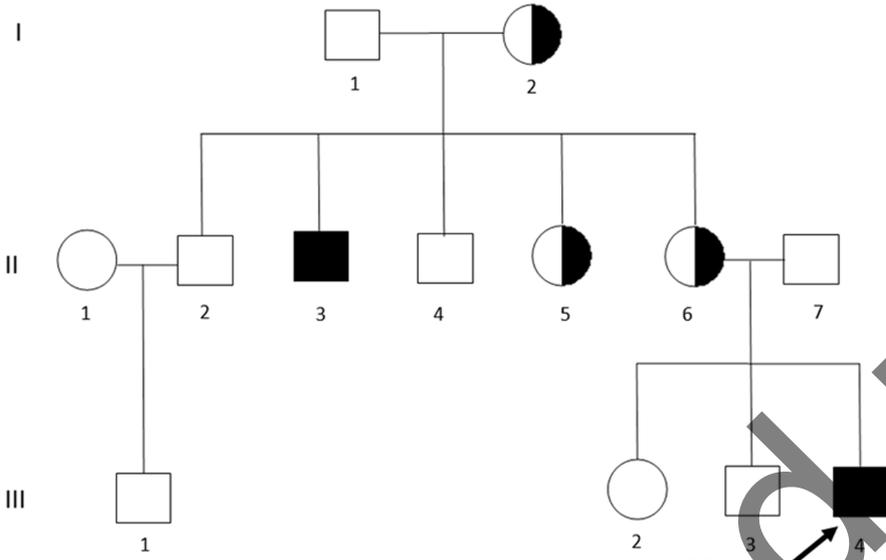


Figure 1. Pedigree of the family. The individuals marked with numbers are those who were available for mutation screening of the *AVPR2* gene. Black and white symbols represent clinically affected and unaffected individuals, respectively. Arrow represents proband.

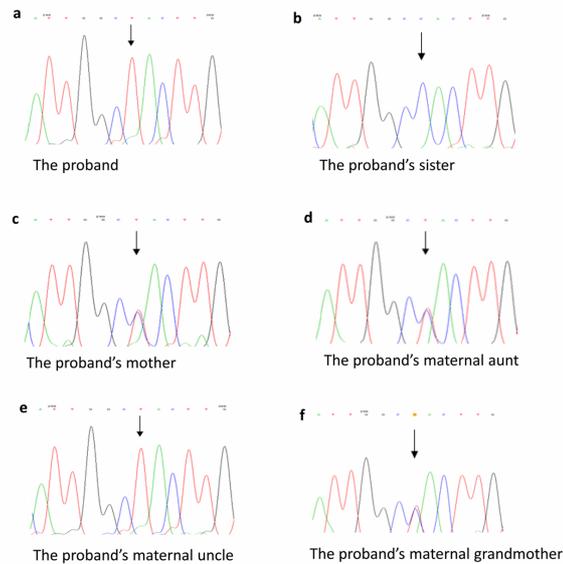


Figure 2. DNA sequencing results from a part of exon 2 of the *AVPR2* gene of proband and his family members. Arrows represent the mutation site.

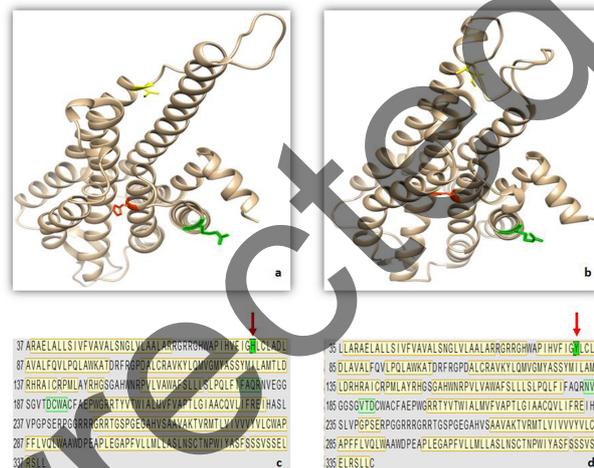


Figure 3. Three-dimensional protein structure predictions of wild-type (a) and mutant (b) AVPR2. Primary structures of wild-type (c) and mutant (d) AVPR2 proteins. Yellow boxes represent α -helix structures and light green boxes represent β -sheet structures. Arrows represent mutation site (processed by UCSF Chimera 1.10.2.).