Case Report

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A novel *AVPR2* gene mutation of X-linked congenital nephrogenic diabetes insipidus in an Asian pedigree

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Abstract

Polyuria and polydipsia are the characteristics of congenital nephrogenic diabetes insipidus (CNDI). Approximately 90% of all patients with CNDI have X-linked hereditary disease, which is due to a mutation of the arginine vasopressin receptor 2 (*AVPR2*) gene. This case report describes a 54-year-old male with polyuria and polydipsia and several male members of his pedigree who had the same symptoms. The proband was diagnosed with diabetes insipidus using a water-deprivation and arginine vasopressin stimulation test. Genomic DNA from the patient and his family members was extracted and the *AVPR2* gene was sequenced. A novel missense mutation of a cytosine to guanine transition at position 972 (c.972C > G) was found, which resulted in the substitution of isoleucine for methionine at amino acid position 324 (p.I324M) in the seventh transmembrane domain of the protein. The proband's mother and daughter were heterozygous for this mutation. The novel mutation of the *AVPR2* gene further broadens the phenotypic spectrum of the *AVPR2* gene.

Keywords

AVPR2, nephrogenic diabetes insipidus, missense mutation

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Introduction

Congenital nephrogenic diabetes insipidus (CNDI) is a relatively rare hereditary disease, which is commonly diagnosed by

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characteristic symptoms, such as polyuria, polydipsia, fever with unknown aetiology, convulsions, vomiting and constipation in early infancy. CNDI is caused by a mutation of the arginine vasopressin receptor 2 (*AVPR2*) gene or the aquaporin 2 gene (*AQP2*) gene.[1,2] Mutation of the *AVPR2* gene leads to X-linked NDI (X-NDI),[3] which accounts for 90% of all diagnosed CNDI cases.[4]

The *AVPR2* gene was first identified in 1992.[1] *AVPR2* maps to chromosome X (Xq28), and thus CNDI is transmitted in an X-linked recessive mode (OMIM 304800);[5] males with the mutated gene are symptomatic, whereas heterozygous females are usually asymptomatic. The AVPR2 protein contains seven membrane-spanning helices.[1] Upon binding with arginine vasopressin (AVP), activation of the receptor is initiated, and allosteric structural rearrangements occur.[6] AVP binds to the AVPR2 within the transmembrane helices II–IV.[7,8] Mutations in the *AVPR2* gene can affect the binding between AVP and AVPR2 or signal transduction, which prevents the renal tubules from concentrating urine and produces the symptoms of polyuria and polydipsia.

The current report presents a case of a Chinese pedigree with CNDI that has a novel missense mutation detected through sequence analysis of the *AVPR2* gene.

Case report

A 54-year-old man, presenting with left limb weakness for 1 week, was admitted to the Department of Neurology, Tianjin Medical University General Hospital, Tianjin, China, on 24 October 2014 with the preliminary diagnosis of cerebral infarction. Dot diffusion-weighted magnetic resonance imaging (MRI) of the head showed high signal intensity, and a softened lesion was found in the right side of the basal ganglia region. The patient had complained of polydipsia and polyuria since birth. He drank 10–15 l of water per day, and his urine output was large as well with nocturia for 5–6 times per night. The patient had no recent history of using renal injury agents. His family history showed his grandmother and grandfather had a consanguineous marriage (they were first cousins). Seven individuals in this pedigree have the same symptoms of polydipsia and polyuria. His younger brother died from dehydration when he was an infant. Because of his seniority and siblings sharing the same symptoms, he had not paid attention to the disease, and received no diagnosis or treatment.

Physical examination showed that his blood pressure was 140/100 mmHg, with a resting heart rate of 94 beats per min, and a body mass index of 26.45 kg/m^2. The myodynamia of his left side of the body was level III. Other examinations showed no abnormalities. Serum sodium and chloride levels were increased above normal values (152 mmol/l and 113 mmol/l, respectively), with an effective osmolality of 310 mOsm/kg.H_2O (normal range: 280–310 mOsm/kg.H_2O). Other serum electrolytes were normal with a serum potassium of 4.04 mmol/l and a serum calcium of 2.53 mmol/l. His hepatic and renal function were normal with a serum albumin of 43 g/l, urea nitrogen of 3.2 mmol/l, and creatinine of 44 μmol/l. The urine specific gravity (1.003) and urine osmolality levels (97 mOsm/kg.H_2O) were decreased compared with normal values (normal osmolality range: 600–1000 mOsm/kg.H_2O), with a total urine volume output of 12.0 l/day. The MRI of his pituitary gland showed an empty sella turcica. Thyroid and adrenal function were normal. An ultrasound of the urinary tract showed mild hydronephrosis of both kidneys and bladder overfilling of approximately 800 ml. Based on the clinical symptoms and laboratory tests, diabetes insipidus was suspected and a water-deprivation and arginine vasopressin stimulation test was performed (Table 1). The test results were compatible with NDI. Combined with the pedigree

Table 1. Water-deprivation and arginine vasopressin stimulation test on a 54-year-old man with suspected diabetes insipidus.

			Urine parameters			
Time, h	BP, mmHg	HR, bpm	Volume, ml	Specific gravity	Osmolality, mOsm/kg.H_2O	Serum osmolality, mOsm/kg.H_2O
0	147/96	93		1.002	91	307
2	145/97	95	2100	1.000		
4	145/95	98	1900	1.003		
5	145/95	98	700	1.002		
6	140/90	115	600	1.002	150	321
7	110/85	120	700	1.003	159	325
8	136/94	102	500	1.002	179	327

Desmopressin acetate (5U) was administered 7 h after the basal study. The test was terminated at 1 h after administration of desmopressin because the patient could not stand the thirst.
BP, blood pressure; HR, heart rate; bpm, beats per minute.

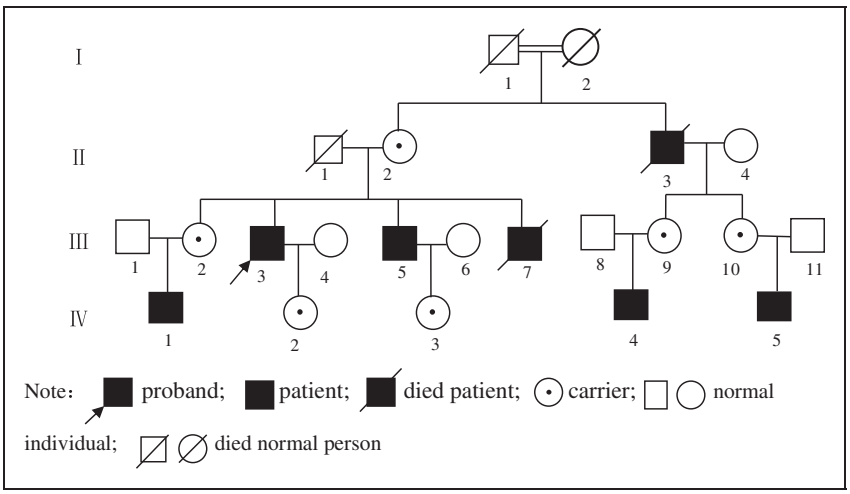

Figure 1. The pedigree map of the proband who was a 54-year-old man with suspected diabetes insipidus.

analysis (Figure 1), the primary diagnosis of X-linked hereditary NDI was made. In order to make a molecular diagnosis, DNA sequencing analysis was carried out on samples from the patient, his wife, four first-degree relatives (his mother, brother, sister, and daughter) and two second-degree relatives (his niece and nephew). His other second- and third-degree relatives refused to have genetic testing, therefore they were not included.

Genomic DNA was extracted from white blood cells. Polymerase chain reaction (PCR) and DNA sequencing were performed following standard protocols. The sequences of the PCR primers are listed in Table 2. All PCR reactions were carried out using the

Table 2. Polymerase chain reaction primers used to amplify four exons of the arginine vasopressin receptor 2 gene.

Primers	Sense	Antisense
Exon 1	5′-TCCGCACATCACCTCCA-GGCC-3′	5′-CCACTTCCTGGCTCCTAGCAGA G-3′
Exon 2	5′-GTCTCTCCAGGCTGCCA-ATGAGTG-3′	5′-CAATCCAGGTGACATAGGTC-3′
Exon 3	5′-CATCTTCGCCCAGCGCA-ACGT-3′	5′-CCTCTAGAGGCAAGACACCCA-ACAGCTCC-3′
Exon 4	5′-CACGTCTTCATTGGCCA-CTTGTGC-3′	5′-CTGGCATGAATCTCCCG-GAAGAT-3′

Thermo Fisher™ Applied Biosystems™ GeneAmp™ PCR System (PCR Master Mix; Tiangen Biotech, Beijing, China). The cycling programme involved preliminary denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 64°C for 60 s, and elongation at 68°C for 120 s, followed by a final elongation step at 72°C for 7 min. The PCR products were separated by 1.0% and 1.5% agarose gel electrophoreses. Phenol/chloroform-extracted PCR fragments were sequenced directly according to standard methodology using an automated sequencer (3500 Dx Genetic Analyzer; Applied Biosystems, Foster City, CA, USA).

Analysis of the *AVPR2* gene sequences of the proband, his brother and nephew revealed a novel missense mutation at coding position 972 (c.972C > G) (Figure 2). This mutation resulted in a change of the 324th amino acid from isoleucine into methionine (p.I324M). His mother, sister, niece and daughter were detected as heterozygotes for the same mutation. His wife was not identified as having an *AVPR2* mutation. The male offspring of the proband's female cousins refused to undertake genetic testing, so were not analysed.

To assess the severity of the mutation *in silico*, the mutation was analysed using Polymorphism Phenotyping v2,[9,10] which predicts the possible impact of an amino acid substitution on the structure and function of a human protein using physical and evolutionary comparative considerations. The software predicted the mutation's effect with a score of 0.998 (out of 1.0), sensitivity of 0.18 and specificity of 0.98, therefore making p.I324M a probable pathogenic mutation.

The study was conducted in accordance with the principles of good clinical practice and the Declaration of Helsinki and was approved by the Ethics Committee of Tianjin Medical University General Hospital. Written informed consent was obtained from all participants for publication of this case report and the accompanying images.

Discussion

In this present study, a pedigree from a consanguineous marriage had several individuals who had experienced polydipsia and polyuria since their infancy. Based on family histories, clinical symptoms (polyuria and polydipsia), laboratory tests (serum sodium, urine specific gravity, urine and serum osmolalities), and the response to a water-deprivation and arginine vasopressin stimulation test, the proband was diagnosed with NDI, and an X-linked recessive CNDI pedigree was established. Further *AVPR2* gene sequence analysis identified a novel missense mutation at coding position

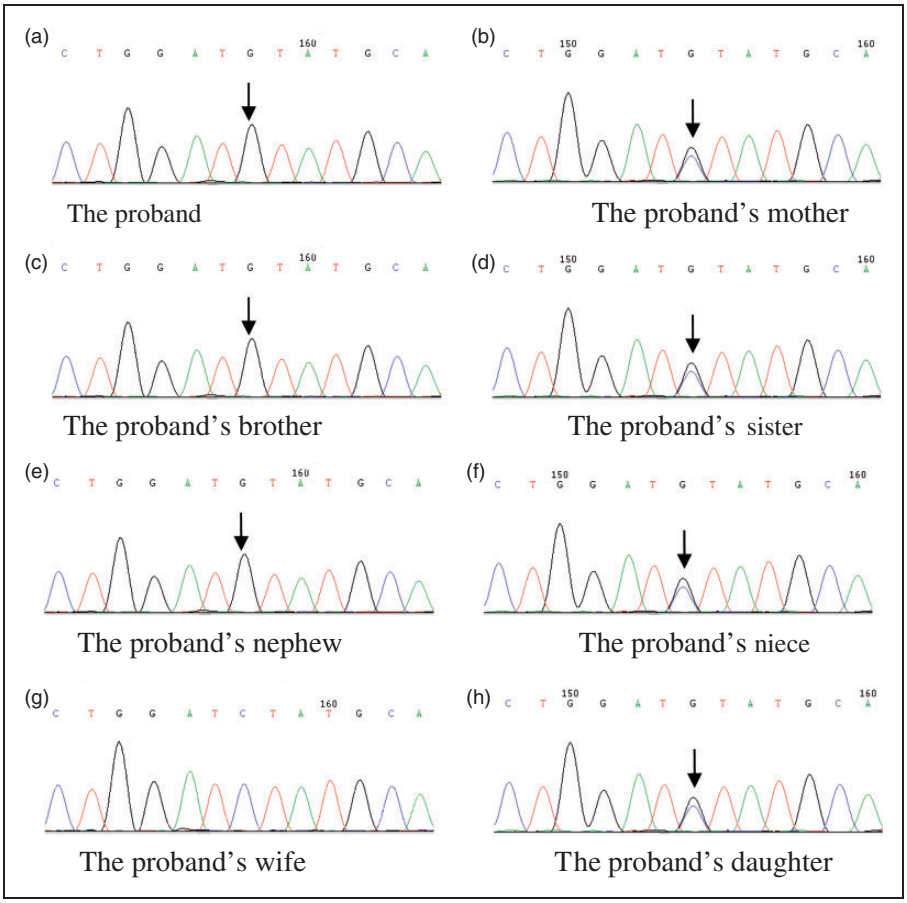

Figure 2. DNA sequencing results for the 54-year-old man with suspected diabetes insipidus and seven first- and second-degree relatives. Arrows represent the mutation site. The colour version of this figure is available at: http://imr.sagepub.com.

972 (c.972C > G), which has not been reported before.

Most patients with CNDI are usually identified in infancy due to their persistent polyuria, severe dehydration, frequent fever, delayed growth, a condition that was first described by McIlraith in 1892. New born infants with NDI often suffer from dehydration, hypernatraemia and seizures, and if it is not diagnosed in a timely manner, those symptoms can be life threatening. In the present study, the proband had experienced a long-term fever during infancy for which an explanation was never found. His younger brother died from dehydration and because several individuals with the same symptoms existed in his family, he did not pay close attention to the disease. The females of the proband's pedigree did not have the symptoms of polydipsia and polyuria. DNA sequence analysis of the proband's mother, sister, niece and daughter showed that they were heterozygous carriers of the mutation. Due to the presence of a normal allele in these females, they had no symptoms of polydipsia or polyuria. All of the patients in this pedigree with symptoms of polydipsia or polyuria were men, which

can be explained by the fact that males have only one X chromosome. Female heterozygous carriers with NDI symptoms can be explained by an extremely skewed inactivation of the normal allele of the X chromosome,[11] but the prevalence of symptomatic female heterozygotes remains unknown.

To date, according to the Human Gene Mutation Database,[12] about 258 mutations in the *AVPR2* gene causing NDI have been identified, including missense mutations, nonsense mutations, splicing mutations and frameshift mutations leading to a premature stop codon.[13,14] Missense mutations are the most common mutated type.[15] An *AVPR2* mutation can cause abnormal protein folding after mRNA translation.[15] *In vitro* experiments have established that most *AVPR2* mutations lead to the encoded receptors being trapped inside the endoplasmic reticulum.[16] A few copies of the mutated receptors can reach the cell surface, but the AVP binding and the Gs protein coupling are impaired, which results in failure to trigger the intracellular cascade of adenylate cyclase effectively.[17] The novel mutation reported by this present study maps to the seventh transmembrane domain of the protein.

One limitation of this present study was the lack of functional studies of the mutated gene. Missense mutations in this region (Trp323, Ger329) have been reported to cause NDI in European populations.[18,19] These studies highlighted the sequence conservation and functional importance of this region.[18,19]

In conclusion, this present case report describes a CNDI pedigree with an *AVPR2* gene I324M missense mutation. To the best of our knowledge, this is the first report of this mutation in patients with CNDI and broadens the phenotypic spectrum of *AVPR2* mutations. Recently, new drugs were found to treat CNDI,[2,20] so identifying *AVPR2* gene mutations may be helpful for specific treatment.

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Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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