Identification of a Novel Arginine Vasopressin Receptor 2 Mutation (p.V183M) in a Chinese Family with Nephrogenic Diabetes Insipidus

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Keywords

Arginine vasopressin receptor 2 · Nephrogenic diabetes insipidus · Novel mutation

Abstract

Loss of function of arginine vasopressin receptor 2 (AVPR2) may affect the recognition and binding of arginine vasopressin (AVP) which, in turn, may prevent the activation of Gs/adenylate cyclase and reduce the reabsorption of water by renal tubules and combined tubes. Finally, the organism may suffer from nephrogenic diabetes insipidus (NDI), a kind of kidney disorder featured by polyuria and polydipsia, due to a break of water homeostasis. In this study, we enrolled a Chinese family with polyuria and polydipsia. The proband presented abnormal fluid intake and excessive urine output. A water deprivation and AVP stimulation test further indicated that this patient had NDI. By sequencing known causative genes for diabetes insipidus, we identified a novel mutation in *AVPR2* (c.547G>A; p.V183M) in the family. This mutation, located in a conserved site of AVPR2 and predicted to be disease-causing by informatics programs, was absent in our 200 controls and other public databases. Our study not only further confirms the clinical diagnosis, but also expands the spectrum of *AVPR2* mutations and contributes to genetic diagnosis and counseling of patients with NDI.

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Nephrogenic diabetes insipidus (NDI) is a rare kidney disorder related to water homeostasis. The main symptoms of NDI are polyuria and polydipsia, but some patients are also observed with vomiting, retching, unexplained fevers, lethargy, and irritability in early infancy [Bockenhauer and Bichet, 2015; Milano et al., 2017; Christ-Crain et al., 2019]. In comparison, acquired NDI is almost always less severe than the hereditary forms [Christ-Crain et al., 2019]. According to previous studies, most inherited NDI (approximately 90%) had an X-linked recessive pattern of inheritance, resulting from arginine vasopressin receptor 2 (*AVPR2*) mutations [Bichet and Bockenhauer, 2016]. In addition, autosomal recessive and autosomal dominant patterns of inheritance can both be present in NDI cases due to mutations in aquaporin 2 (*AQP2*) [Bichet and Bockenhauer, 2016; Milano et al., 2017]. Similarly, variants in arginine vasopressin (*AVP*) and wolframin ER transmembrane glycoprotein (*WFS1*) can also lead to familial diabetes insipidus [Scherthaner-Reiter et al., 2017; Christ-Crain et al., 2019].

AVPR2 is located in Xq28 and encodes the vasopressin receptor 2 which belongs to the 7-transmembrane-domain G protein-coupled receptor (GPCR) superfamily and cou-

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Fig. 1. a Pedigree of the family affected with X-linked dominant inherited NDI. Family members are identified by generations and numbers. Squares indicate male family members; circles, female members; black symbols, the affected members; open symbols, unaffected members; half black, the heterozygous carriers; arrow, proband. **b** Sequencing results of the *AVPR2* mutation. Sequence

chromatograms indicate a G to A transition of nucleotide 547. **c** Analysis of the mutation and protein domains of *AVPR2* and alignment of multiple *AVPR2* protein sequences across species. The affected amino acid V183 is located in the highly conserved amino acid region in different mammals (from Ensembl). Red letters show the V183 site.

ples to Gs, thus stimulating adenylate cyclase [Milano et al., 2017; Kavanagh and Uy, 2019]. *AVPR2* is expressed in the kidney tubule, especially in the distal convoluted tubule and collecting ducts [Grassmeyer et al., 2017]. The main function is to respond to the pituitary hormone AVP by stimulating mechanisms that concentrate the urine and maintain water balance in the organism [Milano et al.,

2017]. At present, more than 200 variants have been identified in NDI patients [Moeller et al., 2016].

In this study, we enrolled a Chinese NDI family with 2 patients and 7 healthy individuals. Genetic investigation and bioinformatics analyses suggested that a novel mutation in *AVPR2* (c.547G>A; p.V183M) may be the genetic lesion of the family.

Table 1. Results of the water deprivation and arginine vasopressin stimulation test

Time, h	Weight, kg	Heart rate, beats/min	Blood pressure, mmHg	Urine volume, mL	Urine specific gravity	Osmolality, mOsm/kg H ₂ O	Serum osmolality, mOsm/kg H ₂ O
0	41	79	125/74	–	1.0005	67	325
2	41	81	120/70	500	1.0005	73	–
4	40.5	97	129/77	450	1.0005	90	–
6	40	122	117/83	300	1.0005	99	–
8	39.5	138	115/70	300	1.0005	105	–
10	39.5	84	110/68	250	1.0006	127	–
12	39.5	89	110/68	100	1.0008	154	355
13	40	88	120/74	350	1.0007	150	353
14	40	84	120/75	250	1.0007	109	320

Desmopressin acetate (5 U) was administered by subcutaneous injection 12 h after the basal investigation.

Material and Methods

Subjects

A 13-year-old boy was admitted to the Department of Nephrology, Third Xiangya Hospital, due to abnormal fluid intake and excessive urine output. In the past year, his water intake had increased from 3–4 L per day to 5–7 L per day, and urine output was more than 10 times per day. The patient reported that only liquid (water and milk) could appease thirst in his infancy. Family history investigation indicated that his uncle (II-1) also suffered from polyuria (5–6 times per night) and polydipsia (10–15 L per day) (Fig. 1a). The proband also accepted the water deprivation and arginine vasopressin stimulation test. Meanwhile, peripheral blood samples of 6 family members were enrolled (II-3 refused to participate).

Mutation Detection and Cosegregation Analysis

Genomic DNA was prepared from peripheral blood of the patients and all other participants using a DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). The entire coding regions including the flanking intronic sequences of *AVPR2* (Refseq: NM_000054), *AQP2* (NM_000486), *AVP* (NM_000490), and *WFS1* (NM_001145853) were amplified by PCR (primer sequences are available upon request). Sequences of the PCR products were determined using the ABI 3100 Genetic Analyzer (ABI, Foster City, CA) as previously described [Fan et al., 2019].

Bioinformatics Analysis

The multiple *AVPR2* protein sequences across mammals were aligned using the program MUSCLE (version 3.6, <http://www.ncbi.nlm.nih.gov>). MutationTaster (www.mutationtaster.org) [Schwarz et al., 2014], SIFT (Sorting Intolerant From Tolerant, <https://sift.bii.a-star.edu.sg/>) [Ng and Henikoff, 2003], and PolyPhen-2 (polymorphism phenotyping, <http://genetics.bwh.harvard.edu/pph2/>) [Adzhubei et al., 2010] were used to predict the effect of the variant. The 1000 Genomes project (1000G, www.1000genomes.org), dbSNP132 (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), ExAC (<http://exac.broadinstitute.org/>), and GenomAD databases (<https://gnomad.broadinstitute.org/>) were used to exclude the polymorphism sites.

Results

Clinical Data

Physical examination revealed that blood pressure (125/74 mmHg), heart rate (79 beats/min), and skin turgor were normal in the proband. Laboratory data were as follows: urine specific gravity 1.0005; uric acid 466.3 μ mol/L; HDL 0.97 mmol/L; Na 146.3 mmol/L; P 1.69 mmol/L; Mg 1.01 mmol/L; glycated hemoglobin 5.3%; normal thyroid function. The results of the water deprivation and arginine vasopressin stimulation test are shown in Table 1. During the water withdrawal period and after injection of desmopressin acetate, the urine specific gravity of the patient did not increase significantly (<1.010). The highest urine osmotic pressure was 154 mOsm/kg, which was much lower than normal osmotic pressure (280–310 mOsm/kg). In addition, urine specific gravity and urine osmotic pressure of our patient did not improve after treatment by desmopressin acetate injection, which led to the hypothesis he might be a NDI patient.

Genetic Analysis

We then examined several genes known to cause diabetes insipidus. By sequencing analysis of *AVPR2*, *AQP2*, *AVP*, and *WFS1*, a novel mutation in *AVPR2* (c.547G>A; p.V183M) was identified which cosegregated with the affected family members (Fig. 1b). This novel mutation, resulting in a substitution of valine by methionine, was absent in our 200 healthy controls [Xiang et al., 2014] as well as in the 1000 Genomes project, dbSNP132, ExAC, and GenomAD databases. Further bioinformatics analyses revealed that this mutation is located in a conserved site

of the third extracellular domain (Fig. 1c) and is predicted to be deleterious by 3 different algorithm-based bioinformatics programs (MutationTaster, SIFT, and PolyPhen-2). Genetic analysis indicated that this novel mutation (c.547G>A; p.V183M) is the genetic lesion of the NDI family. According to ACMG guidelines, this mutation belongs to PS3+PM1+PM2 [Richards et al., 2015].

Discussion and Conclusion

According to previous studies, mutations in *AVPR2* can lead to X-linked recessive NDI. Males with *AVPR2* mutations are symptomatic, whereas heterozygous females are usually asymptomatic [Celebi Tayfur et al., 2018]. However, some heterozygous female carriers have been reported to have NDI; this may be due to random inactivation of the X chromosome [Zhang et al., 2019]. By binding *AVPR2*, AVP can activate Gs/adenylate cyclase and then regulate the water channel AQP2 in renal tubules and collecting ducts, and finally the concentration of urine [Caletti et al., 2014]. In this process, loss of *AVPR2* function may induce a function disorder of the renal tubules and collecting ducts and thus break the water balance of the body [Erdelyi et al., 2015].

In our study, only 2 male family members (II-1 and III-3) presented with NDI phenotypes, the proband's mother (II-5) and his cousin (III-1) were heterozygous carriers and were asymptomatic. The novel *AVPR2* mutation (c.547G>A; p.V183M) is located in the third extracellular domain, which is responsible for AVP recognition and binding [Joshi et al., 2017]. This mutation may disrupt the structure of the extracellular domain and reduce the recognition and binding of AVP, and finally break the water homeostasis and lead to NDI. Our study is consistent with a previous study that mutations in the *AVPR2* third extracellular domain can result in NDI [Joshi et al., 2018]. Similar mutations located in this domain have been reported in NDI patients, such as p.R181C and p.S187R [Pan et al., 1992; Boson et al., 2006].

At present, the treatment of NDI focuses on relieving symptoms, ensuring normal growth and development, preventing complications, and reducing adverse drug reactions [Di Iorgi et al., 2015]. Traditional treatment methods include drinking water, intravenous infusion, and avoiding water poisoning caused by heavy drinking. For underage patients, low-salt diet and reasonable protein diet are necessary to maintain growth and development [Bockenhauer and Bichet, 2015]. The traditional medicine for NDI are thiazide diuretics and a high concentra-

tion of desmopressin [Konoshita et al., 2004; Sung and Lin, 2011; Schernthaner-Reiter et al., 2016]. In our study, the proband accepted the hydrochlorothiazide (25 mg/day) treatment, and the urine volume reduced to 2500 mL per day.

In summary, we report a novel mutation in *AVPR2* (c.547G>A; p.V183M) in a Chinese family with suspected NDI. Our analyses not only further confirmed the clinical diagnosis in the patients, but also expand the spectrum of *AVPR2* mutations and contribute to genetic diagnosis and counseling of patients with NDI.

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Statement of Ethics

The study was approved by the third Xiangya Hospital of the Central South University Ethics Committee and was performed according to the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

J.-S.L. and H.H. carried out the sample collecting and genetic testing. J.-Y.J. and R.D. collected the clinical data. C.-Y.W. performed the bioinformatics analysis. J.-S.L. and L.-L. F. designed the project and wrote the manuscript, and L.-L.F. revised the manuscript. All authors read and approved the final manuscript.

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