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Nephrogenic diabetes insipidus results from a novel in-frame deletion of *AVPR2* gene in monozygotic-twin boys and their mother and grandmother

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Abstract

Objectives: Mutations in the *AVPR2* gene are the most common cause of nephrogenic diabetes insipidus (NDI). In-frame deletions of the *AVPR2* gene are a rare variant that results in NDI. We report a novel variant of the p.H138del in an NDI family with twin male patients and three female carriers of different clinical phenotypes.

Methods: The proband's blood genome was sequenced with a panel, and the variants were classified according to ACMG/AMP (2015) guidelines. X chromosome inactivation (XCI) was analyzed in the peripheral blood of his mother, grandmother, and maternal aunt, respectively. The haplotypes of the X chromosome were determined using their STR loci.

Results: A novel in-frame deletion in the *AVPR2* gene was detected in monozygotic-twin boys, and his mother, grandmother, and maternal aunt were heterozygous carriers. The two boys showed typical NDI, and their mother and grandmother presented polydipsia, polydipsia, and polyuria, but the maternal aunt did not have similar symptoms. The blood XCI results of the mother, grandmother, and maternal aunt showed random inactivation (36.18 , 48.37, and 49.30 %, respectively). The X haplotype indicated that the variant of the mother and grandmother was on their activated X chromosomes(Xa), while the maternal aunt's variant was on her inactivated X chromosome(Xi).

Conclusions: In-frame deletion of the *AVPR2* gene within its functional domain can significantly affect protein function, which is one of the vital causes of NDI. The clinical variability of female carriers of *AVPR2* is associated with underlying environmental and epigenetic factors or complex recombination of the X chromosomes.

Keywords: *AVPR2* gene; nephrogenic diabetes insipidus; X chromosome inactivation; X chromosome recombination

Introduction

X-linked nephrogenic diabetes insipidus (XL-NDI, OMIM: 304800) is a water unbalance disorder. More than 90 % are caused by mutations in the *AVPR2* gene located in Xq28 [1]. The *AVPR2* gene encodes arginine vasopressin receptor 2 (V2R), which is distributed in the renal collecting ducts and plays a role in maintaining the body's water balance [2]. The main characteristics of XL-NDI patients are polydipsia, polyuria, and dysphoria. The main clinical phenotypes include macrocystis, polyuria, irritability, diabetes insipidus, intellectual deficiency, seizures, inability to grow, unexplained fever, polydipsia, hypertonic dehydration, vomiting, constipation, hypernatremia, neonatal-onset, short stature, and feeding difficulties in infancy.

NDI is a rare X-linked recessive genetic disorder. Although the exact prevalence of NDI is unknown, it is estimated that in Quebec, Canada, there are 8.8 cases per 1 million men [3]. Due to the founder effect, certain areas, such as Utah and Nova Scotia have seen an increased incidence [4]. The mutation is typically passed from the mother to her male offspring, leading to a much higher incidence in males. Female carriers exhibit clinical heterogeneity due to varying X-chromosome inactivation statuses [5].

A thorough analysis and identification of the genetic variant have significant clinical implications, especially in managing and counseling affected families. NDI patients should receive timely treatment and management. The water

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intake should not be restricted, and using normal saline (0.9 % NaCl) to treat dehydration can pose serious risks. Heterozygous women with this X-linked condition may be asymptomatic or may experience varying degrees of polyuria and polydipsia. If a pathogenic variant of the *AVPR2* gene was identified in an affected family member, conducting molecular genetic testing on at-risk, asymptomatic female relatives can offer valuable insights into their genetic status. Genetic counseling is essential, as it involves discussions about potential risks to offspring and available reproductive options for affected patients, heterozygotes, and young adults who may carry the risk of becoming heterozygotes.

Here, we present identical twin boys with typical NDI who carried a novel in-frame hemizygous deletion. This research aims to evaluate the pathogenesis of the variant, find the cause of clinical heterogeneity of female carriers, and provide adequate genetic counseling for them.

Materials and methods

Subject

The proband is an eight-month-old boy weighing 7.4 kg when he was hospitalized due to a series of NDI symptoms, including polydipsia, hypernatremia (165 mmol/L), hyperchloremia (122 mmol/L), hypoosmotic urine, low fever, vomiting, and growth and development delay. Ultrasonography revealed a separation of the left pyelon. His identical twin brother also exhibited the same symptoms of NDI. The patients were replenished with adequate fluids and treated with vitamin B6, hydrochlorothiazide, and indomethacin. The twins were born via cesarean section at 36 + 2 weeks, with a weight of 2.2 and 2.3 kg. Furthermore, their mother and grandmother also presented suspected NDI symptoms of polydipsia and polyuria. The parents denied any consanguinity.

Genomic DNA extraction

The genomic DNA was extracted from blood samples of the family using the Qiagen extraction kit. The extracted DNA has a minimum concentration of 20 ng/μL, and the OD260nm/OD280nm ratio falls within the optimal range of 1.8–2.0.

Sequencing and the variants analysis

The proband's blood genomes underwent paired-end sequencing with a panel of renal tubular disease and metabolic disease. The reads were compared to the human reference genome using BWA, Samtools, and Picard software. Mutations were detected using GATK software and annotated using Annovar, FLASH ANALYSIS software. Variants were filtered at 5 % or higher in the GnomAD, ESP, 1,000 Genomes database, and the local healthy population database. Non-functional gene variants, synonymous and non-coding variants, were excluded. Pathogenicity prediction was conducted using SIFT, Polyphen2, M-CAP, and MutationTaster. Candidate variants were analyzed in alignment with the clinical phenotype and family sequencing data while referencing renowned databases and literature sources such as OMIM, HGMD, ClinVar, MITOMAP, and PubMed. Furthermore, copy number variations (CNVs) were checked using ReadDepth. Finally, the variants were confirmed by Sanger sequencing. The variants were classified according to ACMG/AMP (2015) guidelines [6].

The X chromosome inactivation analysis

The capillary electrophoresis identified four polymorphic genetic markers loci (XpA, XpB, XpC, and XqA) according to the manufacturer's instructions(Shanghai Cubicise Medical Co., Ltd). The multiple PCR amplification conditions were as follows: 94 °C for 2 min; then 26 cycles of (98 °C for 10 s, 62 °C for 60 s, 68 °C for 60 s); followed by 72 °C for 10 min. The XCI was determined by calculating the peak-height ratios of alleles, including before and after the process of restriction enzymes Hpa II. The electrophoresis was carried out using the ABI 3500Dx gene analyzer, and the data were analyzed using GeneMapper software 5.0. The XCI calculation method and judgment criteria were the same as in the previous report [7].

Analysis of other STR loci on the X chromosome

Short tandem repeat (STR) was detected using fluorescent PCR plus capillary electrophoresis. The STR loci, including DXS9902, DXS7132, IDXq, GATA165B12, DXS1187, DXS6809, DXS8377, and DXS981, were analyzed with the X chromosome multi-STR genotyping kit from Jingzhun Corp. and Darui Corp., respectively. The amplification conditions were as follows: 95 °C for 15 min, 94 °C for 30 s, 58 °C for 1 min and 30 s, 72 °C for 1 min and 30 s, for 27 cycles, and a final extension at 72 °C for 30 min. The amplified products were then analyzed by capillary electrophoresis using an AB 3500Dx gene analyzer, and the electrophoresis data were processed using GeneMapper software.

The X chromosome haplotype and X recombination analysis

The X chromosome haplotypes of the family were constructed with 12 STR loci, including XpA, XpB, XpC, XqA, DXS9902, DXS7132, IDXq, GATA165B12, DXS1187, DXS6809, DXS8377, DXS981. The X recombinations of the mother and maternal aunt were deduced from the STR of their parents.

Results

Sequencing results

The proband was identified with a novel hemizygous variant of the *AVPR2* gene (chrX: 153171373–153171375, NM_000054; exon3, c.413_415delACC(p.H138del). Family verification confirmed that the proband's twin brother shared the same hemizygous variant, and the mother, grandmother, and maternal aunt were all found to be heterozygous, while the wild type in father and grandfather. For more details, please refer to the Sanger sequencing diagram (Figure 1) and family diagram (Figure 2).

The results of X chromosome inactivation

The analysis of X chromosome inactivation revealed that the blood XCI of the mother, grandmother, and maternal aunt were randomly inactivated at a rate of 36.18 , 48.37, and 49.30 %, respectively, as shown in Figure 2 and Figure 3.

The results of X chromosome haplotype and X recombination analysis

The proband and his identical twin brother inherited an allelic variant from their mother. The X haplotype results showed that the proband's mother and grandmother had the variant on each active X chromosome(Xa), while the maternal aunt's on her inactivated X chromosome(Xi). The X-chromosome haplotype structure of the mother and maternal aunt revealed several recombinations between the grandmother's homologous X chromosomes and an additional Xqters exchange from the grandmother and grandfather in the maternal aunt's X chromosomes. For more details, please refer to Figure 4.

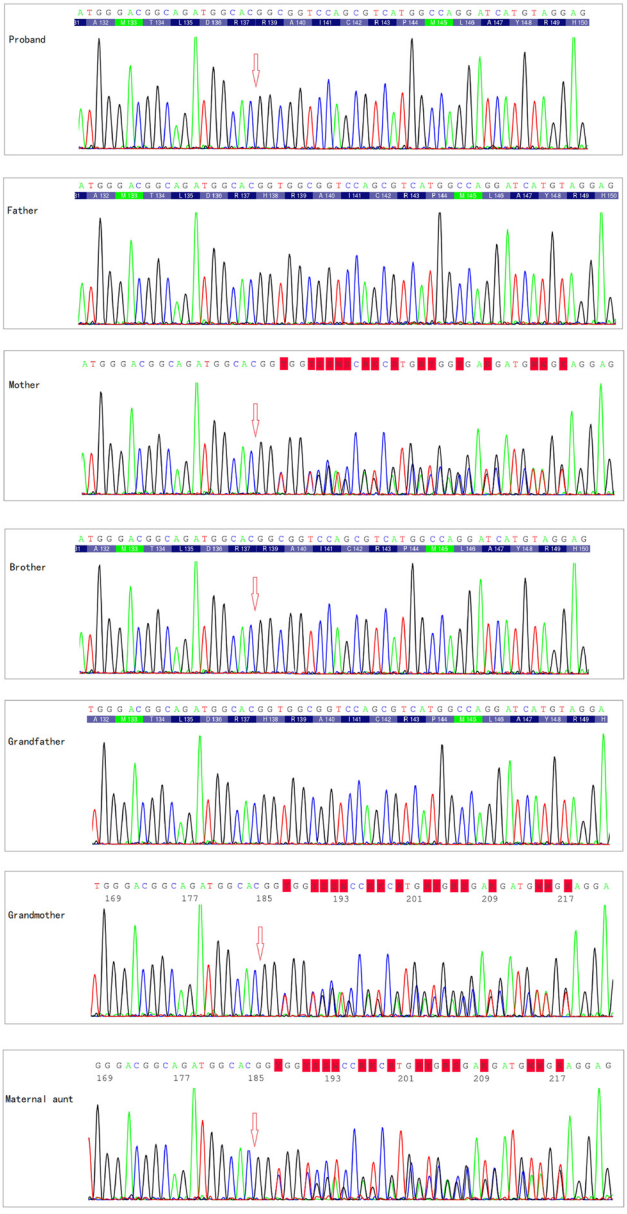

Figure 1: Sanger sequencing of *AVPR2* gene. The proband and twin brother shared the same hemizygous variant, and the mother, grandmother, and maternal aunt were all found to be heterozygous, while the wild type in father and grandfather. Arrows indicated the variants.

In-frame deletion variants of the *AVPR2* gene related to NDI

Some in-frame deletion variants of the *AVPR2* gene are associated with NDI, as detailed in Table 1. These NDI cases involved the deletion of single or multiple amino acids. We identified the p.H138del mutation in the *AVPR2* gene, located in the IC-2 domain, in twins who exhibit severe NDI-related

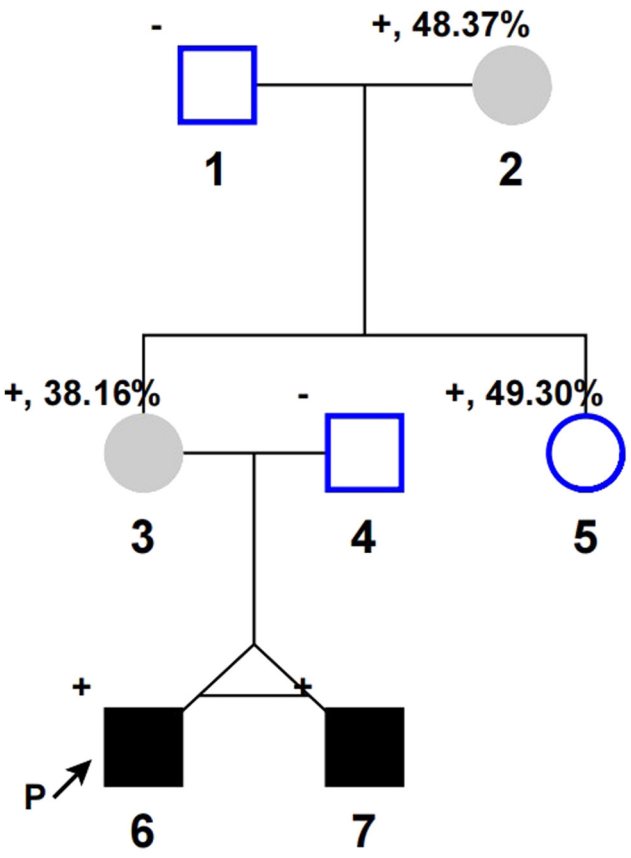

Figure 2: Family diagram of *AVPR2* gene variant. (-): Wild type; (+): Mutant type; (%): X chromosome inactivation ratio; (→): The proband.

phenotypes. Among the 21 reported cases of in-frame deletions in the *AVPR2* gene – comprising two cases from our study and 19 others documented in the literature and the HGMD database – various domains were affected, including intracellular (IC), extracellular (EC), transmembrane (TM), as well as the NH3 and COOH end. Out of these 21 cases, four had unknown clinical phenotypes. Among the remaining 17 cases classified as typical NDI, two were mild and involved the TM domain, while three were severe, affecting the IC and EC domains.

Discussion

In this study, both the male proband and his identical twin sibling showed typical symptoms and signs of NDI, such as polydipsia, hypernatremia, hyperchloremia, hypotonia, growth delay, repeated hypothermia, and vomiting. And they shared a hemigamous mutation of *AVPR2* exon3 (NM_000054) c.413_415delACC(p.H138del) (GRch37 chrX: 153171372–153171375). HGMD has reported more than 300 variants of the *AVPR2* gene result in NDI. Most *AVPR2* mutations of NDI are missense mutations, followed by nonsense mutations, and fewer are in-frame deletions. However, the latter two may cause more serious diseases [8]. Bicchet et al. [2] showed that the *AVPR2* receptor protein has seven transmembrane domains, connecting three extracellular domains (EC-1, EC-2, EC3) and three intracellular loops (IC-1, IC-2, IC-3). In HGMD, several cases of NDI with in-frame deletion of *AVPR2* have been reported, such as CD2115497: 380_382delCCT (Ser127del), CD001867: 382_384delTAC(Tyr128-del), CD001870: 526_528delTTC (Phe176del), CD983639: 606_608delTCG(Arg203del). Meanwhile, previous studies reported some NDI cases of *AVPR2* in-frame deletion, including single or multiple amino acids. For details, please see Table 1. The clinical phenotypes of some patients were mild, and others were severe. The severity might vary with the different domains involved in deletion. In our patient, c.413_415delACC (p.H138del) is an in-frame deletion of a single amino acid. Although there still have been no familiar cases reported, p.H138 is in an important functional domain, IC-2, which is highly conserved in different species [14]. We speculated the variant might affect the protein function. According to the population frequency, highly-specific phenotype, hereditary mode, the variant is classified as likely pathogenic.

The grandmother, mother, and maternal aunt all had the heterozygous variant, and we have clarified their clinical variability. Compared to women without the variant, the grandmother and mother experienced significant increases in both take-in water and urine volume, along with frequent nighttime urination, 3 to 4 times per night. They often felt tired and thirsty, preferred cold drinks, experienced easy fatigue, and occasionally felt sleepy. Additionally, they faced menstrual irregularities, while the aunt did not exhibit any of these symptoms. Although only about 1 % of women with the *AVPR2* heterozygous variant have the same severe phenotype as men with NDI [3], a higher proportion of female carriers have significant NDI-related symptoms. In a Japanese study, 25 % of female carriers developed NDI [20], and a Spanish study showed a 50 % incidence of female carriers [21]. Like 75 % of genes on the X chromosome, the *AVPR2* is a gene subject to X inactivation [22]. The clinical phenotype of female individuals carrying the same *AVPR2* heterozygous variant ranges from severe to mild or even normal phenotype, is associated with variations in underlying environmental and epigenetic factors or differences in complex recombination of the X chromosomes. At first, XCI is the well-known influence factor. The different XCI statuses varied with age and tissue [5, 22–25]. In our study, preferential X inactivation was not shown in the peripheral blood of the mother and grandmother with NDI clinical phenotype, consistent with the previous reports that blood lymphocytes of female NDI patients with random X inactivation [24, 26].

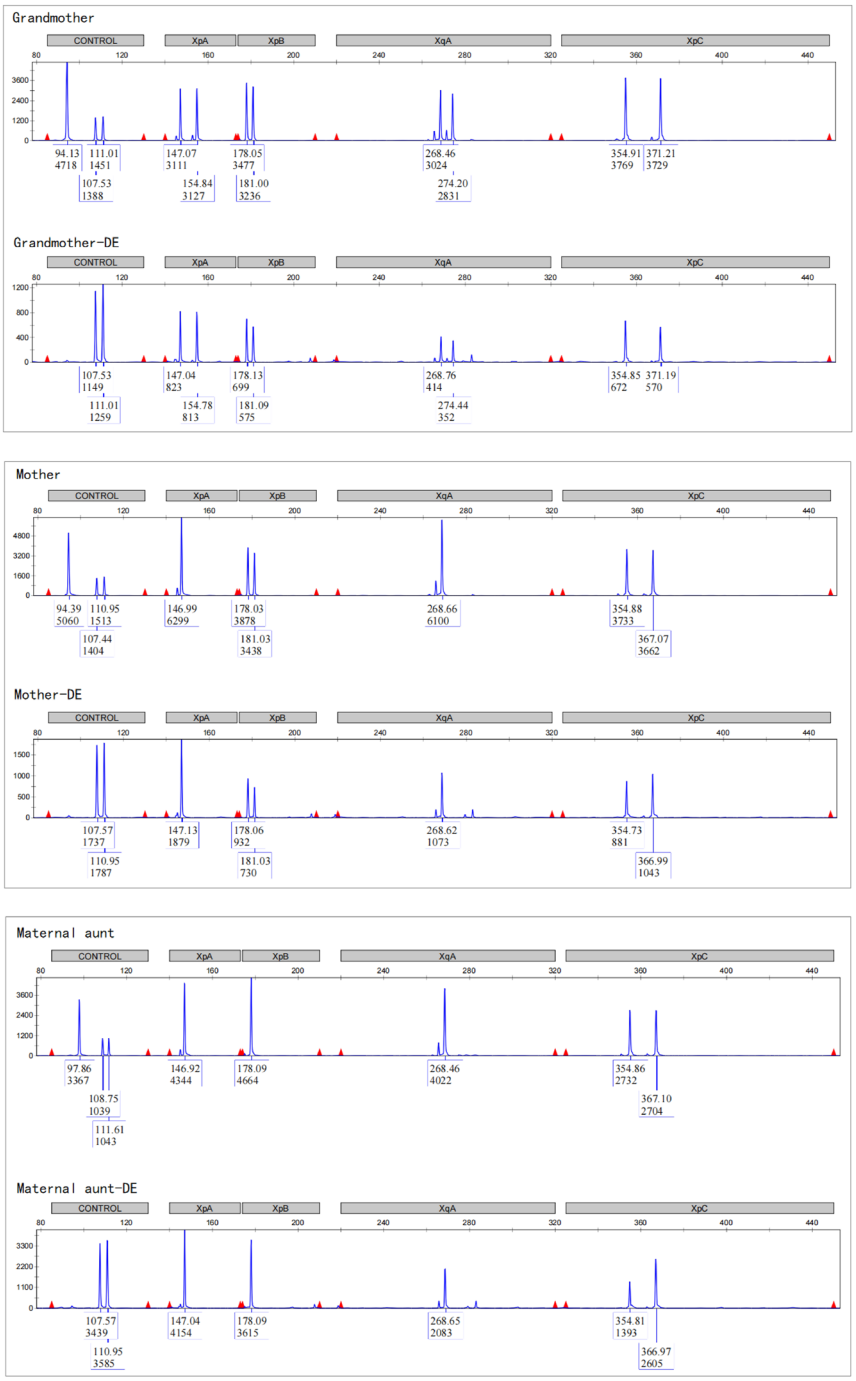

Figure 3: The electrophoretogram of blood XCI in the family. The vertical and horizontal axes represent fluorescence intensity and fragment length, respectively, with the corresponding values below the fluorescence peak. Three control fragments were analyzed from left to right to evaluate enzyme digestion efficiency, PCR amplification, and the copy number of the X chromosome. Before digestion, heterozygous genetic markers, including XpA, XpB, XpC, and XqA, were used to determine the status of X chromosome inactivation (XCI). The allele with the highest fluorescence peak after digestion is labeled D1, while its corresponding undigested peak is labeled U1. The other alleles are designated as D2 and U2, respectively. To calculate the XCI rate, we use the formula: (d1/u1)/(d1/u1+d2/u2) *100 %. A bias in XCI is indicated if the XCI ratio exceeds 75 %. The results of XCI revealed that the blood XCI of the mother, grandmother, and maternal aunt were randomly inactivated at a rate of 36.18 , 48.37, and 49.30 %, respectively. DE: digestation of enzyme.

Although X chromosome inactivation (XCI) levels in their blood tissues appeared randomly inactivated, XCI levels may vary, particularly in renal collecting ducts cells. Given the severity of their clinical phenotypes, we hypothesize that the renal collecting ducts cells of the mother and grandmother may have skewed XCI, whereas the aunt likely has random XCI. Unfortunately, renal collecting ducts cells samples from family members are unavailable for further testing. Secondly, X chromosome recombination or exchange is another factor affecting clinical heterogeneity. The X-chromosome haplotype structure of the mother and maternal aunt revealed several recombinations between the grandmother's homologous X chromosomes and an additional Xqters exchange from the grandmother and grandfather in the maternal aunt's X chromosomes. The recombination rate of the X chromosome is much higher than that of autosomes [27]. Recombination factors should also be considered in X-chromosome-related diseases, the same as in the study of

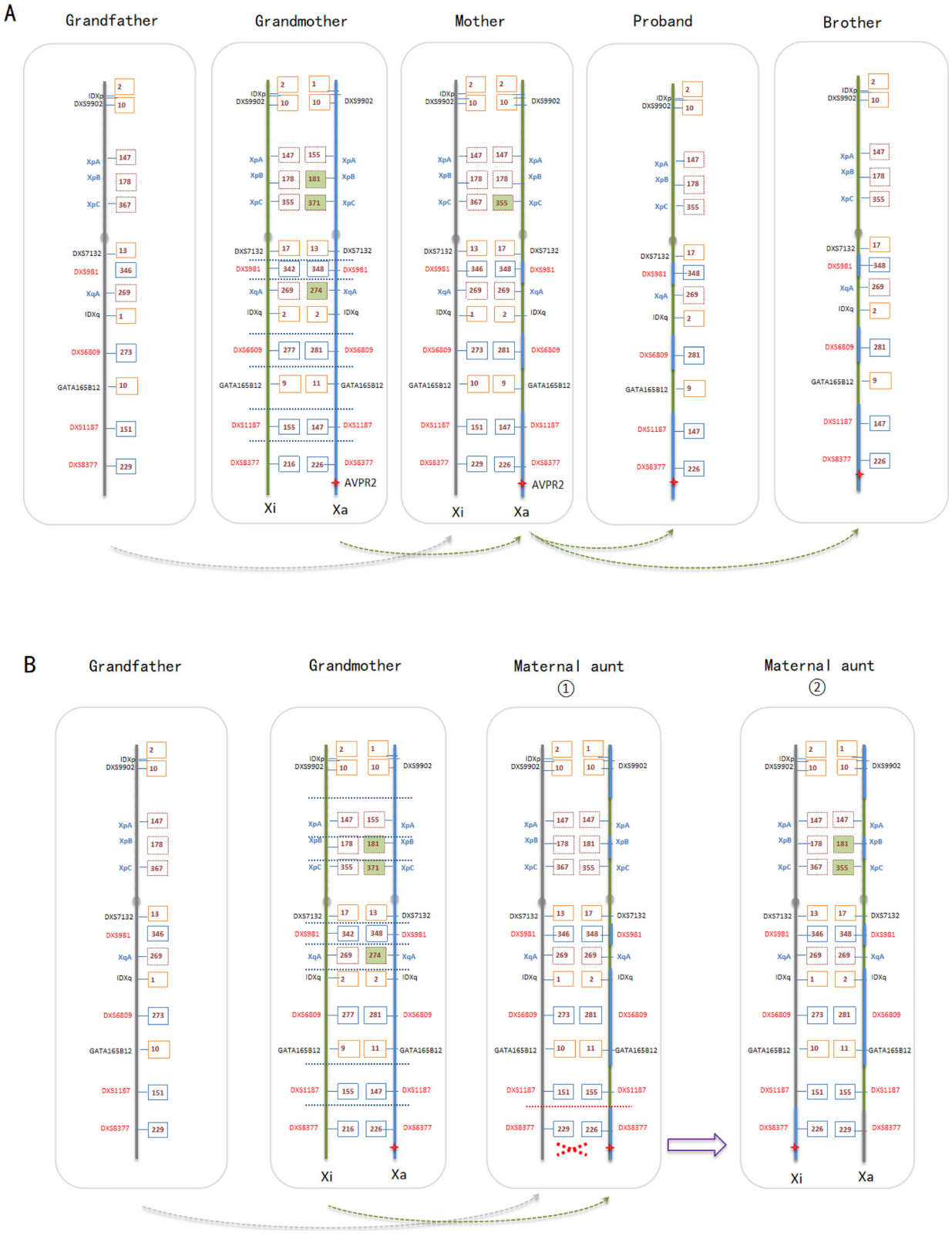

Figure 4: The diagram of X chromosome haplotype and X recombination of the family. (A) The proband and his twin brother had identical short tandem repeat (STR) loci, and both inherited an allelic variant of the *AVPR2* gene (marked with a red star) from their mother and the mother from her mother. The variant of the grandmother and mother was on each active X chromosome(Xa), indicated by the green box. (B) The *AVPR2* variant of the maternal aunt was on the inactivated X chromosome(Xi). Her X chromosome haplotype hinted several recombinations from her mother's X-homologous chromosomes (the blue dashed line indicated the recombination breakpoints) and additional Xqters exchange (red dashed line and red cross) from her parents.

Table 1: In-frame deletion variants of the *AVPR2* gene related to NDI.

Patient sex	Variant site (GRCh37/hg19)	HGVS (nucleotide) (NM_000054)	HGVS (protein)	Exon	Domain position	Reported phenotype	References
Male	chrX:153,171,373-153,171,375	c.413_415delACC	H138del	3	IC-2	NDI, severe	This study
Male	chrX:153,171,373-153,171,375	c.413_415delACC	H138del	3	IC-2	NDI, severe	This study
Male	chrX:153,171,069–153,171,071	c.109-111delGCC	A37del	3	NH3 end	NDI	ELIAS et al. (2008) [8]
Male	chrX:153,171,144–153,171,146	c.185_187delCTA	L62del	3	IC-1	NDI	ELIAS et al. (2008) [8]
Male	chrX:153,171,189-153,171,191	c.229_231delTTC	F77del	3	IC-1	NDI	HGMD: CD2111641
Male	chrX:153,171,339-153,171,341	c.380_382delCCT	S127del	3	TM	NDI	HGMD: CD2115497
Male	chrX:153,171,342-153,171,344	c.382_384delTAC	Y128del	3	TM	Unknown	Arthus et al. (2000) [3]
Male	chrX:153,171,486-153,171,488	c.526_528delTTC	F176del	3	TM	Unknown	Arthus et al. (2000) [3]
Male	chrX:153,171,564-153,171,566	c.604_606delCGT	R202del	3	EC-2	NDI, severe	Ala et al. (1998) [9]
Male	chrX:153,171,567-153,171,569	c.606_608delTCG	R203del	3	EC-2	NDI	HGMD: CD983639
Male	chrX:153,171,699-153,171,701	c.742-744delCGC	R247del	3	IC-3	NDI	ELIAS et al. (2008) [8]
Male	chrX:153,171,795-153,171,797	c.835_837delGTC	V279del	3	TM	NDI, mild	Arthus et al. (2000) [3], Shoji et al. (1998) [10], Schulz et al. (2002) [11], Tsukaguchi et al. (1993) [12], Wildin and Cogdell (1999) [13], Wildin et al. (1998) [14]
Male	chrX:153,172,012–153,172,014	c.946_948delCTC	L316del	4	TM	NDI	HGMD: CD2045252
Male	chrX:153,172,066–153,172,068	c.999_1001delCTC	S334del	4	COOH end	NDI	HGMD: CD120242
Male	chrX:153,171,069–153,171,092	c.109_132delGCCCGGGCGGAGCTGGCGCTGCTC	A37_L44del	3	TM+NH3 end	Unknown	Gu et al. (2002) [15]
Male	chrX:153,171,144–153,171,152	c.184_192delTAGCTCGGC	L62_R64del	3	IC-1	NDI	Bichet et al. (1994) [16], HGMD: CD941606
Male	chrX:153,171,159-153,171,167	c.200_208delGGCGGGGCC	R67_G69del	3	IC-1	NDI	HGMD: CD1211229
Male	chrX:153,171,213-153,171,224	c.255_263delCCTGGCCGTinsGAG	D85_V88delinsGlu	3	TM	NDI	Chen et al. (2020) [17]
Male	chrX:153,171,279-153,171,296	c.318_336delGGGCCAGATGCCCTGTGT	G107_C112del	3	EC-1+TM	NDI, mild	Owada et al. (2002) [18]; HGMD: CD025453

Table 1: (continued)

Patient sex	Variant site (GRCh37/hg19)	HGVS (nucleotide) (NM_000054)	HGVS (protein)	Exon	Domain position	Reported phenotype	References
Male	chrX:153,171,699-153,171,710	c.739_750delCGCCGCAGGGGA	R247_250G	3	IC-3	Unknown	Bichet et al. (1993) [19]
Male	chrX:153,171,705-153,171,716	c.744_755delCAGGGGACGCCG	R249_R252del	3	IC-3	NDI	HGMD: CD920835

NDI, nephrogenic diabetes insipidus; IC, intracellular domain; EC, extracellular domain; TM, transmembrane domain.

kinship [28]. Previous studies have estimated that the recombination rate of PAR1 is 20 times the genomic average [29], and that of PAR2 is five times [30]. Although the recombination rate of PAR2 is much lower than PAR1, it is still higher than the average recombination rate of the rest parts of the X chromosome [31]. In addition, non-homologous recombination at the pseudoautosomal boundary of X is also a common situation [29]. The *AVPR2* gene was only about 1.80 Mb away from the PAR2 region, so we speculated the *AVPR2* gene exchange of the maternal aunt was related to the PAR2 region. When the PAR2 boundary region was recombined or exchanged, the maternal *AVPR2* variant transferred into another Xi, which inactivated the variant *AVPR2* gene, presenting no NDI phenotype. On the contrary, the *AVPR2* variant of the grandmother and mother was on the Xa, and their mutation effects showed up.

Our study compares initially the clinical severity of the novel in-frame deletion variants of the *AVPR2* gene identified here with those reported previously. We found that the inconsistencies in clinical severity among these variants may be related to their involvement in different domains. In future studies, we will further investigate the impact of these variants on gene function and conduct additional functional assessments.

XCI is one of the pivotal clinical reference indicators for some X-linked diseases in female carriers. Several issues should be in mind in XCI testing. Firstly, we should choose the appropriate method of XCI detection. The methylation status of a polymorphic locus in the androgen receptor gene (HUMARA assay) [32, 33] is a popular method. The STR heterozygosity of the AR gene in the population is about 90 % [34, 35], and the remaining 10 % homozygous locus of female individuals cannot be detected. Therefore, the application range of this method in scientific research and clinical practice is limited. In this study, four polymorphic genetic marker loci, including the AR gene, were simultaneously subjected to methylation-sensitive restriction enzyme digestion. According to the kit instructions, the detection rate is as high as 99 %, with at least one locus as heterozygous. Secondly, the related tissue or functional cells should be detected when evaluating the XCI status. Although both peripheral blood and urinary system cells are of mesodermal origin, we cannot predict the clinical phenotype of female carriers by evaluating the XCI of peripheral blood leukocytes instead of renal collecting ducts cells, which affect urine reabsorption. Thirdly, the recombination of sex chromosomes is frequent, so haplotype analysis of X chromosomes should be performed when analyzing X-related diseases. When adopting the X-STR marker, it is crucial to consider linkage disequilibrium [28, 36], as the STR of the X chromosome shows more serious linkage disequilibrium than those on autosomes [36–38].

Primary manifestations and complications can be prevented through timely diagnosis and clinical intervention in suspected NDI patients during their early life [39]. Patients need lifelong treatment. Treatment methods mainly include general symptomatic treatment and individualized drug treatment: the former limited sodium and unlimited water intake, adequate care, and sufficient nutrition, and the latter hydrochlorothiazide and indomethacin, and so on. Meanwhile, we recommend enhanced surveillance and management for female carriers. During pregnancy, heterozygous women with *AVPR2*-NDI may experience a slight increase in urine production and thirst. In a small number of pregnancies involving fetuses affected by NDI, excessive amniotic fluid may occur, necessitating frequent drainage to alleviate discomfort for the mother, particularly in cases of severe hyperamnios [40]. If pathogenic variants of the *AVPR2* gene are identified in affected family members, conducting molecular genetic testing on asymptomatic at-risk female relatives is highly beneficial. Maternal aunts of male proband may be at risk of being heterozygous carriers of pathogenic variants, and their offspring may also be at risk for male patients or heterozygosity.

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Research ethics: This study was approved by the Sichuan Provincial Women's and Children's Hospital/The Affiliated Women's and Children's Hospital of Chengdu Medical College Institutional Review Board (20230331-054).

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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Conflict of interests: The authors state no conflict of interest.

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Data availability: Not applicable.

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