

# Integrating population variation and protein structural analysis to improve the clinical genetic diagnosis and treatment in children with congenital nephrogenic diabetes insipidus

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## Research

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# Abstract

**Background and Objectives:** Congenital nephrogenic diabetes insipidus (NDI) is a rare genetic disorder characterized by renal inability to concentrate urine. Establishing the genetic diagnosis appears particularly important to NDI for early detection and differential diagnosis.

**Method:** We utilized a Chinese multicenter registry to investigate genotype and phenotype in children with NDI from 2014 to 2019. The structural locations of the pathogenic mutations from this study and the literature, as well as population variants retrieved from gnomAD were analyzed.

**Results:** A total of 10 boys from 9 families carried mutations in *AVPR2* (8/10) or *AQP2* (2/10). Another 7 relatives of the families were diagnosed by sequencing for partial or subclinical NDI. Patients presented with dehydration, polyuria-polydipsia, and severe hypernatremia with a median age at diagnosis of 1.0 month (IQR 0.16, 18). Protein structural analysis revealed a notable clustering of diagnostic mutations in the transmembrane regions of *AVPR2*, and enrichment of diagnostic mutations by autosomal dominant inheritance (AD) in the C terminal region of *AQP2*. The pathogenic mutations are significantly more likely to be buried inside the domain comparing the population variants. Through structural analysis and in silico prediction, the eight mutations identified in this study were considered as presumably disease causative. The most common treatments were thiazide diuretics and non-steroidal anti-inflammatory drugs (NSAIDs). Emergency treatment of hypernatremic dehydration in neonates should not choose the isotonic saline as a rehydration fluid.

**Conclusion:** Genetic analysis presumably confirmed the diagnosis of NDI in every patient of the studied cohort. A plea of early identifying NDI confirmed by phenotype and genotype, and consequently optimize the treatment.

## Introduction

Congenital form of nephrogenic diabetes insipidus (NDI) a rare inherited disorder, characterized by insensitivity of the distal nephron to the antidiuretic action of arginine-vasopressin (AVP) and the reduced ability of the kidney to concentrate the urine, leading to severe dehydration and electrolyte imbalance (hypernatremia and hyperchloremia)<sup>1</sup>. Inheritance is X-linked in 90% of patients due to mutations in the gene coding for the vasopressin type 2 receptor (*AVPR2*) (OMIM #304800)<sup>1,2</sup>. The remaining patients have autosomal recessive or dominant forms due to mutations in the gene coding for the water channel aquaporin 2 (*AQP2*) (OMIM #222000; OMIM #125800)<sup>1,3</sup>. The main clinical hallmarks of NDI are polyuria and compensatory polydipsia. Upon inadequate water supply, a hot environment, or episodic losses of free water, patients suffering from NDI do not properly compensate water loss and are at risk of severe dehydration. The urine concentrating defect is present at birth, and symptoms arise during the first week of life as irritability, poor feeding, and failure to thrive<sup>4</sup>. Persistent polyuria can lead to the development of kidney megacystis, hydroureter, and hydronephrosis. Repeated episodes of dehydration can cause mental retardation<sup>5</sup>, which is a serious complication of NDI, probably secondary to hypoxic episodes<sup>6</sup>.

Establishing the genetic diagnosis appears particularly important for NDI for early detection and differential diagnosis in view of its unique associated features and long-term complications.

We utilized a multicenter strategy to investigate the genotype and phenotype in a cohort of Chinese children clinically diagnosed with NDI in the current study. The description of clinical and genetic spectrum of Chinese children with NDI will help to devise a strategy for gene analysis appropriate for our population.

## Materials And Methods

### Study design and participants

The national multicenter registry (Chinese Children Genetic Kidney Disease Database, CCGKDD, [www.ccgkdd.com.cn](http://www.ccgkdd.com.cn)) has assembled the largest genetic screening cohort of pediatric renal disease in CHINA to date<sup>7</sup>. Enrolled participants were solicited via clinicians from the CCGKDD. Participants were asked to provide information on presenting clinical features, genetic diagnosis, monitoring, medical management, age at each clinical event and status at last follow-up. Respondents could report details of one or multiple NDI patients. No identifying information was collected about patients or respondents. The name of the reporting center was collected to allow comparison of entries to avoid duplicates. Among the children with renal disease consecutively enrolled in the national multicenter registry CCGKDD from 2014 to 2019, children were diagnosed of NDI based on patient symptoms, biochemistry disturbances genetic testing and/or water restriction/ Desmopressin (DDAVP) loading tests.

### Genetic analysis

In order further identify and confirm the diagnosis, we performed the trio whole exon sequence (Trio-WES) including proband and parents concurrently after the informed consent was obtained from his parents. Samples were subjected to Whole Exome Sequencing (WES) in outsourcing, and raw data were transferred to our lab for the bio-informatic analysis. FastQ raw data were analyzed by FastQC software to check the quality in terms of length and GC content of reads, quality of nucleotides within the reads, over-represented sequences (PCR duplicates or excess of adaptors), etc. Alignment of the sequences in Hg19 genome reference was followed by variant calling, assessed by the Haplotype Caller tool of the newest version of GATK. Variants were annotated for predicted effect on protein function; allele frequency in public databases gnomAD (<https://gnomad.broadinstitute.org/>); and predicted pathogenicity with in silico algorithms, including SIFT (<https://sift.bii.a-star.edu.sg/sift-bin/>), PolyPhen-2 score (<http://genetics.bwh.harvard.edu/cgi-bin/pph2>), MutationTaster (<http://www.mutationtaster.org/cgi-bin/>). Evidence for disease causality was assessed using ClinVar and the Human Genome Mutation Database (HGMD), followed by manual review of the cited primary literature.

### Protein structural analysis

The structural analysis of AVPR2 and AQP2 was performed on PDB accession 6U1N and 4NEF respectively. The utility of Protter (<https://wlab.ethz.ch/protter/start/>) is performed for the integrated

visual analysis of membrane proteins<sup>8</sup>. Residue depth, a computational procedure for calculating the depth of a residue from the protein surface, was calculated using DEPTH server (<http://cospi.iiserpune.ac.in/depth/>)<sup>9</sup>.

Additional disease causative mutations in AVPR2 or AQP2 were identified through HGMD (<http://www.hgmd.cf.ac.uk/ac/all.php>) and ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and a search of published literature. Population variations in AVPR2 or AQP2 was investigated in gnomAD, which are presumed to be benign with respect to the be relatively enriched and pathogenic variants relatively depleted for rare childhood disease. According to the estimated prevalence in PDI males of 8.8: 1,000,000<sup>2</sup>, the variants of allele frequency more than 8.8/1,000,000 were retrieved as population single-nucleotide polymorphism (SNPs). Number of residues involved in variants within the function domains were compared in the population SNPs and the disease causative mutations reported in HGMD or ClinVar.

## Results

### Patient Characteristics

A total of ten boys from nine families with registry information on CCGKDD database from 2014 to 2019 were enrolled in this study. Patient characteristics were shown in Table 1. The median age at clinical diagnosis was 1.0 months (IQR 0.16, 18). There were three cases with delayed diagnosis who were clinically diagnosed after 2 years old of age. One of them was identified the mutation of *AQP2* until developing into chronic kidney disease (CKD) 3 stage at 14 years old. Polyuria-polydipsia and hypernatremia were presented in all the ten probands. Five patients were diagnosed during the neonate period. And four of them had fever, vomiting and anorexia at the initial presentation. Even five families had multiple affects, only one family applied for early genetic screening for the newborn baby because of his affected sibling with NDI. Five patients had a water restriction test before genetic detection. Eight patients underwent cranial MRI as part of their diagnostic evaluation without any abnormalities. Hydronephrosis was found in four children. No patient had hypercalciuria or abnormal renal function checking for the serum chemistries at initial presentation.

Table 1  
Mutations and clinical follow-up of patients with nephrogenic diabetes insipidus

ID	Gender	Age of clinical diagnosis / Age of genetic diagnosis	Family History	Pathogenic Gene	Mutation (Exon / Nucleotide change / Amino acid change)	Initial presentation	Urologic involvement	Urine specific gravity / Urine Volume (ml/kg.h)	Serum Sodium / Urine sodium (mmol/L)	Pituitary gland MRI	Water deprivation test	Treatment / Last follow-up (serum Na: mmol/L, age: yrs)
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1	Male	3.6 yrs / 4 yrs	None	AQP2	exon3:c.559C>T;p.R187C(HOM)	polydipsia, polyuria	None	< 1.005 / 20	159 / 20	normal	negative	Thiazide / 145, 6.5
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

2	Male	3 yrs / 13 yrs	Paternal / paternal grandpa	AQP2	exon 4 c.803delGp.R268Vfs*67 (het, p, het; m, wt)	polydipsia, polyuria	None	< 1.005 / 23	150 / N.D.	N.D.	N.D.	Thiazide / 140, 14.2 #
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.



3	Male	5 days / 2 months	Maternal uncle	AVPR2	exon 2: c.185T > C; p.L62P (Hemi)	jaundice, fever, vomiting, anorexia, Hypernatremia	None	< 1.005 / 12	161 / 54	normal	negative	Thiazide, indometacin / 145, 4.2
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

4	Male	3 days / 26 days	none	AVPR2	exon 1: c.229-231delTTTC; p.F77del (Hemi)	polyuria, fever, vomiting, anorexia	Hydronephrosis	< 1.005	/ 1	0	162/16	normal	N.D.	Thiazide; indometacin / 145, 2.5
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

5	Male	2 months / 2 months	None	AVPR2	exon 2: c.494C>	Fever, vomiting, anorexia	None	< 1.005 / 7	162 / 14	normal	N.D.	Thiazide / 148, 3.0
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

6	Male	2 yrs / 4.1 yrs	None	AVPR2	exon 3: c.500C > T; p.S167L (hemi)	polydipsia, polyuria	None	1.002 / 7	145 / 26	normal	negative	Thiazide; indometacin / 140, 7.5
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

7	M	1	M	AVPR2	exon3:c.500C>	polydipsia, polyuria	None	1.000 / 7.4	145 / 23	normal	negative	Thiazide / 140, 2.2
-	a	y	a		T;p.S167L(hemi)							
1*	l	r	t									
*	e	s	e									
		/										
		1										
		y										
		r										
		s										

All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

7	M	1	M	A	e	p	H	1	1	n	N	T
-	a	4	a	V	x	o	y	.0	4	o	.D	h
2	l	d	t	P	o	n	d	0	9	r	.	i
*	e	a	e	R	3	3	r	0	/	a		a
		/	r	2	:	1	e	/	1	l		z
		1	a		c	-	p	2	2	/		i
		m	t		.9	9	,p					d
		o	e		9	1	o					e
		n	r		1	-	l					/
		t	a		-	9	y					1
		m	t		9	9	u					4
		o	e		2	d	r					2
		n	r		e	e	i					,
		t	a		l	f	a					1
		m	t		G	,f					0	
		o	e		o					5		
		n	r		,f					.		
		h	a		v					5		
		o	t		o							
		n	e		m							
		h	r		i							
		s	a		t							
		2	t		g							
		5	e		,							
		h		a								
		e		n								
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

8	Male	1 month / 2 months	Maternal / maternal grandpa	AVPR2	exon 3 : c . 991 - 992 del GC ; p . S331 Rfs * 25 (hemi)	polydipsia , polyuria , fever , vomiting , anorexia	Hydronephrosis	1 . 003 / 10	160 / 48	normal	N . D .	Thiazide ; indometacin / 145 , 2 . 6
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

9	Male	1 month / 4.6 yrs	Maternal uncle	AVPR2	Exon2del (hemi)	polydipsia, polyuria, Urinary incontinence	Hydronephrosis	1 / 15	135 / 17	normal	Negative	Thiazide / 135, 5.8
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All patients with mutations in the *AVPR2* gene were hemizygous segregated with all mothers were heterozygous for the given mutation.

het, heterozygous; hemi, hemizygous; m,maternal; N.D.no data; p, paternal; \* siblings

# the patient developed into CKD 3 stage at 14 years old.

## Genetic analysis

We detected six different mutations of *AVPR2* and two mutations of *AQP2* in the study cohort. Four of them were previously described mutations (*AVPR2*: p.L62P<sup>10</sup>, p.A165D<sup>11</sup>, and p.S167L<sup>12</sup>; *AQP2*: p.R187C<sup>13</sup>), and four novel mutations were observed (*AVPR2*:p.F77del, p.S331Rfs\*25 and small deletion of exon2; *AQP2*: p.R267fs\*66). In total, four were missense, three were small deletions resulting in frameshift mutations and the last one was a gross deletion of an exon (Table 1 and Figure. 1). Pathogenicity predicted with in silico algorithms was show in Table 2.



Table 2  
Pathogenicity of mutations predicted with in silico algorithms.

	<i>AVPR2</i>					<i>AQP2</i>			
Mutation	p.L62P (Hemi)	p.F77del	p.A165D	p.S167L	p.S167L	p.S331Rfs*25	Exon2 del	p.R187C	p.R268Vfs*67
PPH2 score	1	-	0.763	-	0.993	-	-	0.998	-
SIFT	DAMAGING	-	TOLERATED	-	DAMAGING	-	-	DAMAGING	-
MT	Disease Causing	-	Disease Causing	-	Disease Causing	-	-	Disease Causing	-
HGMD	Knoers (1994) Kidney Int 46, 170 <sup>[10]</sup>	None	Schulz (2002) J Clin Endocrinol Metab 87, 5247 <sup>[11]</sup>	None	Wildin (1994) Am J Hum Genet 55, 266 <sup>[12]</sup>	None	None	Lieburg (1994) Am J Hum Genet 55, 648 <sup>[13]</sup>	None
Ortholog	Dr	-	Mm	-	Gg	-	-	Xt	-
gnomAD (ESA)	1/177848 (0/13463)	None	None	None	None	None	None	15/282878 (0/19954)	none

We also detected seven female relatives of heterozygous mutations and two maternal uncles of hemizygous mutations in the *AVPR2* gene. Among them, we found that three suffered polydipsia and polyuria, and another four female individuals presented partial or subclinical NDI.

## Variants analysis and comparative protein modeling

The AVPR2 protein is a typical seven membrane-spanning helices G protein-coupled receptor (GPCR) localized at the basolateral plasma membrane of the principal cells of the kidney collecting duct<sup>14</sup>. The literature contains 169 reported missense mutations in AVPR2 in patients with NDI, and there are a further 109 population missense variants retrieved from the gnomAD database. The location of disease-causing and population variants was scattered throughout the different structural domains of the protein, with significant enrichment of disease-causing variants in transmembrane region (Fisher test,  $P=0.001$ , Fig. 1A, C). The Residue Depth of the disease-causing mutation is significantly higher than that of the population SNPs and that of all variants from gnomAD (Wilcoxon-Mann-Whitney test,  $P=2e-8$  and  $P=2e-5$  respectively, Fig. 2A,B).

Structural analysis of the AVPR2 revealed the presence of hemizygous mutations in eight patients (Fig. 1A). The three missense mutations of *AVPR2* (p.L62P p.A165D and p.S167L) produce full length misfolded proteins with residues located within the transmembrane domain, mostly retain in the endoplasmic reticulum (ER) by the ER quality-control machinery and target for proteasome degradation. The deletion mutation p.F77del results in the residue deficiency in the first intracellular loop (located in a well-conserved region), probably causes a defective protein. The small deletion p.S331Rfs\*25 causes a frame shift with a premature stop codon encountered in the sequence. This deficiency of C-terminal tail of AVPR2 loses the binding of  $\beta$ -arrestin to the phosphorylated tail of AVPR2 and subsequently receptor internalization allowing the engagement of G protein to the core of AVPR2. The forming of megaplex AVPR2-G protein-  $\beta$ -arrestin is required for the active signals leading to sustained endosomal cAMP generation<sup>15</sup>. The gross deletion of exon 2 leading to truncated proteins, which are often rapidly degraded.

The *AQP2* gene is located on chromosome 12q13 and codes for the 271 amino acid AQP2 protein, a type IV-A transmembrane protein characterized by six transmembrane domains connected by five loops and intracellular N- and C-termini<sup>16</sup>. A total of 53 missense mutations and 9 small deletion in *AQP2* were reported, and there are a further 70 population missense variants retrieved from the gnomAD database. The location of disease-causing and population variants was scattered throughout the different structural domains of the protein, with no significant enrichment of disease-causing variants in transmembrane regions (Fisher test,  $P=0.079$ , Fig. 1B). However, five of nine small deletion in AQP2 affected the residues from 223 to 271 on C terminal cytoplasmic region. There was significantly enrichment of disease-causing variants by autosomal dominant inherited (AD) in the C terminal region (Pearson test,  $P=0.0001$ , Fig. 1D). The Residue Depth of the disease-causing mutation is significantly higher than that of the population SNPs and that of all variants from gnomAD (Wilcoxon-Mann-Whitney test,  $P=2e-7$  and  $P=2e-5$  respectively, Fig. 2C, D).

Structural analysis of the AQP2 revealed the presence of mutations in two patients (Fig. 1B). The homozygous mutation p.R187C affects amino acids in the selectivity filter region of the water conduction pore which determines the transport specificity<sup>17</sup>. ER accumulation of these AQP2 mutants has been shown in several studies<sup>4</sup>. The C terminal truncating mutation p.R267fs\*66 could result in the heterotetramers formed by wild type and mutated AQP2 monomers which either retained in the Golgi apparatus or misrouted to late endosomes, lysosomes, or basolateral membrane. This kind of mutation is inherited from a dominant trait and are causative of autosomal dominant NDI<sup>18</sup>.

## Treatment Regimens

All the babies diagnosed with NDI during the neonate need emergency treatment of hypernatremic dehydration in this study. Most of them received isotonic or hypotonic rehydration before the clinical diagnosis of NDI. Such was observed in our case example ID#3 whose plasma Na increased from 153 to 161 mmol/l after rehydration with intravenous 0.19% saline for the neonate. A tonicity balance can easily demonstrate the excess of NaCl administration with 0.19% saline (Fig. 3A, B). In this case, the appropriate

fluid use of 5% glucose which provides the free water lost was prescribed at maintenance fluid rates appropriate to his age and size, pending adjustment according to the plasma Na. And the priority was breastfeeding for the hypotonic advantage compared with formula feed.

Most of our patients started the conventional treating with thiazides after clinical diagnosis of NDI, followed by non-steroidal anti-inflammatory drugs (NSAIDs). Thiazides were prescribed to all the ten patients, a thiazide and NSAID in four (4/9) patients. During the follow up, NSAIDs were discontinued for the following reasons including general concerns about long-term use (3/4), and increased serum creatinine (1/4). Medium serum sodium at initial treatment was 154 mmol/L (IQR 145, 161). At last follow-up, median age was 5.0 years old (IQR, 2.6, 8.3) and median serum sodium 144 mmol/L (IQR 140, 145) (Fig. 3C). At the time of last follow-up only one case (#2) with *AQP2* mutation developed into CKD 3 stage whose father and maternal grandfather passed away with renal failure.

## Discussion

This study reported the genetic spectrum and treatment approaches in a cohort of children with NDI registered on the Chinese multicenter database. We reported eight cases of seven families presented with X-linked NDI (*AVPR2*), one presented with autosomal recessive NDI (*AQP2*) and one autosomal dominant NDI (*AQP2*).

Although the pathophysiology and molecular diagnosis of congenital polyuric states has been well established<sup>19</sup>, we still encounter cases where the diagnosis is late and where inappropriate diagnostic testing and treatments are done. Optimizing the diagnostic strategy including genetic analysis is one of our top priorities. As is known disease-causing genes for NDI, it has been well established the heterozygous loss of function variants of *AVPR2* or *AQP2* result in congenital NDI<sup>2,3,20</sup>. In order to try to elucidate the pathogenicity of variants of *AVPR2* or *AQP2*, we compared population SNPs with pathogenic mutations from HGMD. Although in principle the existence of a single population variant does not rule out pathogenicity, it is unlikely that the observed population variants of *AVPR2* or *AQP2* are pathogenic, since severe early-onset childhood disorders have specifically been excluded from gnomAD. We evaluated the variants in the 3D domain structure encoded by *AVPR2* or *AQP2* to find the positional correlation with pathogenicity. There is notable clustering in *AVPR2* 3D structure, with pathogenic mutations more likely to be within transmembrane region. Such clustering in transmembrane region was not shown for *AQP2*. An enrichment of diagnostic mutations by autosomal dominant inherited (AD) was found in the C terminal region of *AQP2*. Nonetheless, the pathogenic missense mutations of *AVPR2* or *AQP2* were significantly more likely to be buried within the domain. Systemic analysis of the protein structure and variants allowed us to make strong predictions about likely pathogenic variations both in *AVPR2* and in *AQP2*. One of the limits of our study is the lack of functional studies, especially in case of the novel variants detected in the NDI cohort. So, the mutations identified in our study were considered as presumably disease causative post the protein structural analysis and in silico prediction.

As next generation sequencing is increasingly applied in both research and clinical settings, more and more variants will be discovered in known disease genes as well as in novel disease genes. Although *in silico* predictions alone should not be relied on as the sole basis to determine the clinical significance of variants in proteins, we hope that the analysis used in this study provides useful structural evidence for variants interpretation. Moreover, combining clinical and population genetics with protein structural analysis offers a widely applicable *in silico* method for improving the clinical interpretation of novel missense variation.

Massive free water losses cause significant morbidity, even in treated patients<sup>6</sup>. High fluid intake is necessary to avoid hypernatremic dehydration, which can result in permanent neurologic complications. Most emergency protocols suggest initial treatment with 0.9% saline<sup>21</sup>. While the situation is different in NDI, because of the ongoing losses of essentially pure water with the urine. Infusion of 0.9% saline will result in excess sodium chloride administration and thus worsen the hypernatremia as observed in our case treating with 0.19% saline. Thus, children with NDI should be treated with hypotonic fluids, either enterally (water/milk) or, if needed, intravenously (5% dextrose in water)<sup>6,22</sup>. Of course, hypotonic fluids must never be administered as an intravenous bolus. Emphasis on the infusion rate which only slightly exceeds the urine output is to safely normalize plasma sodium concentration at a rate of less than 0.5 mmol/l/h (10–12 mmol/l/day). The main risk of a rapid decrease in plasma sodium is cerebral edema and potentially death. Yet, the concern of this complication and misunderstanding of NDI would lead to administration of excess salt with a risk of rapid increase in plasma Na, leading to osmotic demyelination<sup>6</sup>. The diagnosis of congenital NDI can either be missed or misunderstood, leading to dangerous mistreatment. It is quite important to early identify NDI with phenotype and genotype, and consequently optimize the treatment.

Hypernatremia in children with NDI will induce a strong thirst behavior and, when asked, the parents of patients with NDI often provide the typical history of an extremely thirsty child, who so avidly drinks large amounts of water, that it often vomits afterward. Older babies will typically want to preferentially drink water and avoid food. Patients are also at risk for nocturnal enuresis, hydronephrosis, and poor growth, presumably due to the need for high water consumption that interferes with adequate caloric intake. In our cohort, five neonates were diagnosed early with symptoms including fever, vomiting and anorexia. Even five families had multiple affecteds, only one family applied for early genetic screening for the newborn baby because of his affected sibling with NDI. Besides the indexes, we identified the three more males diagnosed of NDI by sequencing of *AVPR2* and *AQP2*, and another four female individuals presented partial or subclinical NDI. Confirming the clinical diagnosis with genetic screening allows the early diagnosis and management of at-risk members of families with identified mutations<sup>6,19,23</sup>.

Our study had several limitations. The data were collected retrospectively from the registry system and we were not able to obtain complete data on all of the patients. We described the complications and treatment approaches during a median follow-up period of 5 years. Urological complications were noted such as hydronephrosis and urinary incontinence (4/10). Unfortunately, there were no more details on nutrition, growth and mental development in our registry. It has been reported the long-term morbidities

caused by NDI including primary nocturnal enuresis (44%), persistent small stature (38%), urologic complications (37%), persistent failure to thrive (29%), and CKD stage 2 or greater (30%)<sup>23</sup>. Here we reported only on case with delayed diagnosis who developed into CKD 3 stage.

## Conclusion

New-born and young children with polyuria symptoms should be immediately referred to specialized centers with experience in treating hypernatremic dehydration and the ability to obtain rapidly a clinical diagnosis and genetic study.

## Abbreviations

3D: three dimensions;

AD: autosomal dominant inherited;

AR: autosomal recessive inherited;

AVP: arginine-vasopressin;

AVPR2: vasopressin type 2 receptor;

AQP2: aquaporin 2;

CCGKDD: Chinese Children Genetic Kidney Disease Database;

CKD: chronic kidney disease

DDAVP: Desmopressin;

GPCR: G protein-coupled receptor

HGMD: Human Genome Mutation Database;

IQR: Interquartile range;

NDI: nephrogenic diabetes insipidus;

NSAIDs: non-steroidal anti-inflammatory drugs

OMIM: Online Mendelian Inheritance in Man;

PCR: Polymerase chain reaction;

SNPs: single-nucleotide polymorphism;

Trio-WES: trio whole exon sequence;

WES: Whole Exome Sequencing

## **Declarations**

## **Ethical approval and consent to participate**

All procedure performed in studies involving human participants were in accordance with the ethical standards of the Ethical Committee for scientific research approval of the Children's Hospital of Fudan university. Informed consent Written informed consent was obtained from all patients and their parents for publication.

## **Consent for publication**

The parents of the children and adult patients described in this article provided consent for participation in the study and for publishing the obtained results.

## **Competing interests**

None declared.

## **Availability of data and materials**

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## **Authors' Contribution**

JR designed and supervised the study, wrote the manuscript. PLL and HXL performed clinical examinations, collected blood samples, wrote the clinical part of the manuscript. TCX, YF, JR performed

bio-information evaluation and protein structural analysis; all the authors contribute to the clinical information and registry database; JR, HX, DM critically revised the manuscript

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## Figures



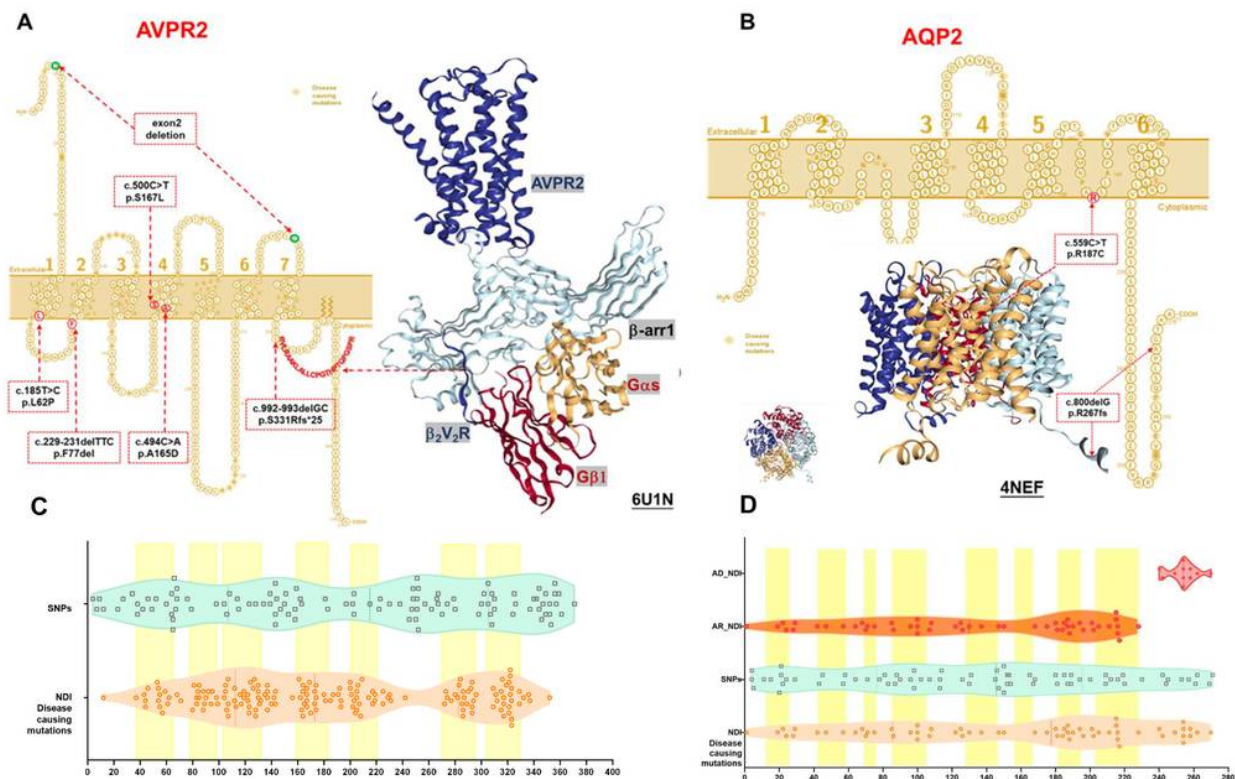
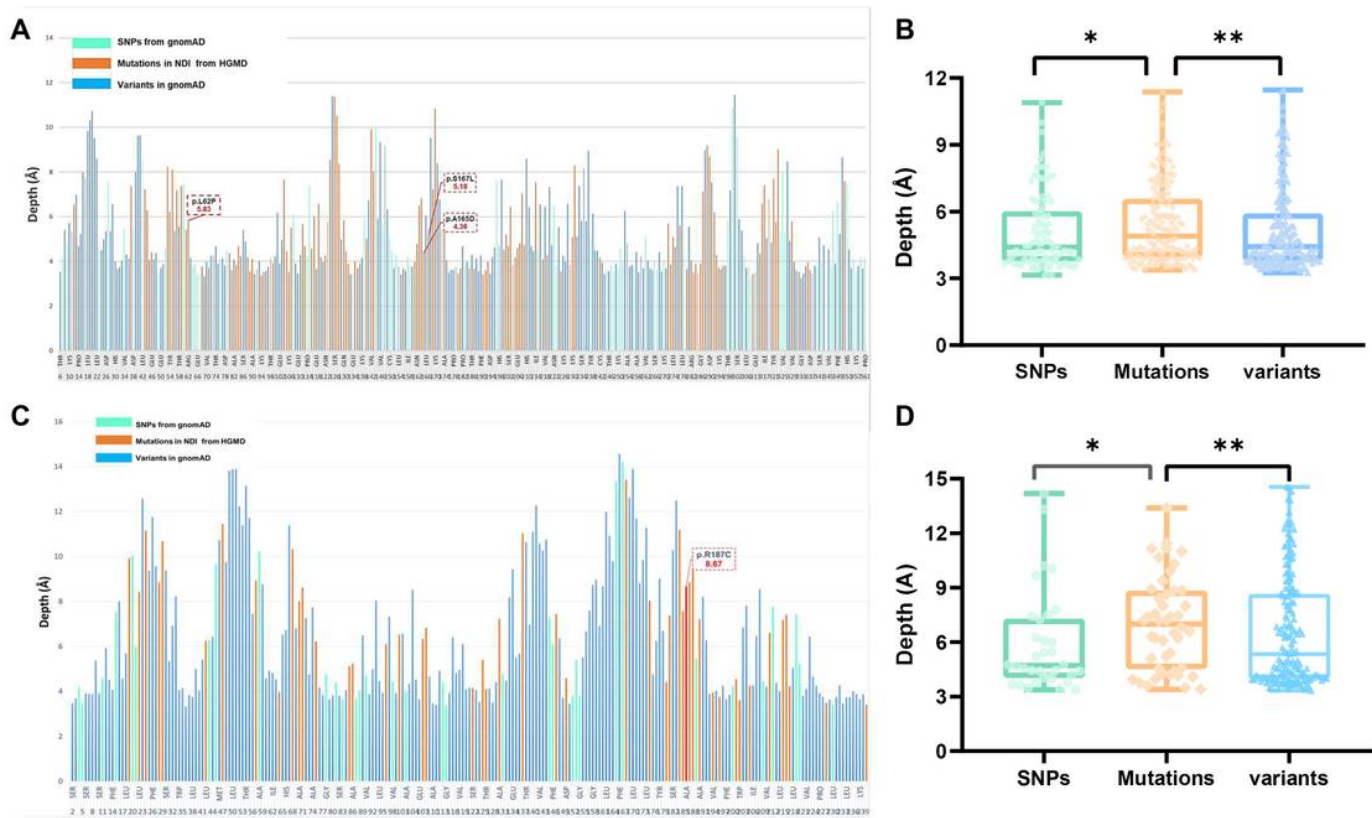


Figure 1. Schematic representation and protein structure of AVPR2 and AQP2 protein with location of mutations.

## Figure 1

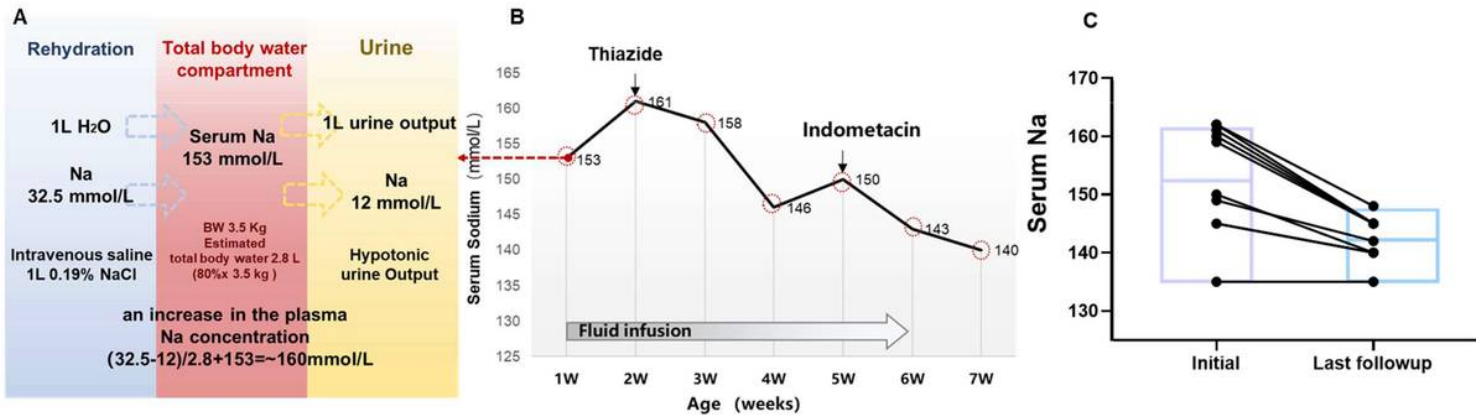
Schematic representation and protein structure of AVPR2 and AQP2 protein with location of mutations. A. AVPR2, which belongs to the G-protein-coupled receptor (GPCR) superfamily, is formed by 371 amino acids with seven transmembrane, four extracellular and four cytoplasmic domains, and is present at the basolateral membrane (UniProtKB P30518-1, P30518-2)<sup>14</sup>. The seven transmembrane domains with the extracellular NH2 terminus and its intracellular COOH terminus are illustrated as originally reported. Ribbon diagram of AVPR2 from PDB entry 6U1N was shown with GPCR- $\beta$ arrestin structure in lipid bilayer. The six mutations identified in our NDI cohort were indicated in red dotted box, and the diagnostic mutations by HGMD were labeled in orange diamond. B. AQP2, which belongs to the larger family of major intrinsic proteins, is formed by 271 amino acids. Aquaporins contain two tandem repeats, each containing three transmembrane  $\alpha$ -helices domains with the amino and the carboxyl termini located on the cytoplasmic surface of the membrane (UniProtKB P41181)<sup>16</sup>. The six transmembrane domains with the intracellular NH2 and COOH terminus are illustrated as originally reported. Ribbon diagram of AQP2 from PDB entry 4NEF was shown with side and top view. The two mutations identified in our NDI cohort were indicated in red dotted box, and the diagnostic mutations by HGMD were labeled in orange diamond. C. A violin plot revealed the domain composition of AVPR2 with the location of the 109 population variants (SNPs) from gnomAD (turquoise square) and the 169 diagnostic mutations from HGMD (peachpuff circle). The seven transmembrane regions were labelled as yellow box. There was a significant enrichment of diagnostic mutations in the transmembrane regions (Fisher test,  $P=0.001$ ). D. A violin plot revealed the domain composition of AQP2 with the location of the 70 population variants (SNPs) from gnomAD (turquoise square) and the 62 diagnostic mutations from HGMD (peachpuff circle)

including 56 mutations inherited by autosomal recessive trait (AR, orange circle) and 6 mutations inherited by autosomal dominant trait (AD, scarlet dot). The eight transmembrane regions were labelled as yellow box. There was no significant enrichment of diagnostic mutations in transmembrane region compared with population variants (Fisher test,  $P=0.079$ ). The mutations by AD were more likely located in the C terminal region compared with that by AR (Pearson test,  $P=0.0001$ ).



**Figure 2**

Analysis of variants and residue depth computation<sup>9</sup> in AVPR2 and AQP2. A. A 2D plot of residue-wise depth of AVPR2 (PDB 6U1N) with bars indicating the diagnostic mutations by HGMD (orange), population variants/SNPs by gnomAD (turquoise) and all the other residues (blue). B. Boxplot of the residue depth comparing in the diagnostic mutations (orange), population variants/SNPs (turquoise) and all the other residues (blue) in AVPR2. \*  $P=2e-8$ , \*\*  $P=2e-5$  (Wilcoxon-Mann-Whitney test) C. A 2D plot of residue-wise depth of AQP2 (PDB 4NEF) with bars indicating the diagnostic mutations by HGMD (orange), population variants/SNPs by gnomAD (turquoise) and all the other residues (blue). D. Boxplot of the residue depth comparing in the diagnostic mutations (orange), population variants/SNPs (turquoise) and all the other residues (blue) in AQP2. \*  $P=2e-7$ , \*\*  $P=2e-5$  (Wilcoxon-Mann-Whitney test)



**Figure 3. Clinical treatment and outcome in 10 children with NDI.**

**A.** Simplified tonicity balance for the neonate ID#3 with NDI, excreting hypotonic urine and receiving 0.19% saline. When considering separately the balances for water and Na, the excess Na administration from 0.19% saline becomes immediately obvious. The red box in the middle represents the total body water compartment of a patient with NDI. A patient with NDI excretes hypotonic urine, detected with the urine Na concentration of 12 mmol/l. If 1 L of urine output is replaced with 1L of 0.19% saline, this will not change the fluid balance, but lead to a net gain of 20.5 mmol of Na. In the neonate of 3.5 kg with estimated 2.8L of total body water, this would lead to an increase in the plasma Na concentration of approximately  $20.5 \text{ mmol} / 2.8\text{L} = 7.3 \text{ mmol/l}$ .

**B.** Serum sodium and treatment in the case #3.

**C.** Follow up of the serum sodium level in ten patients with treatment of thiazides and indometacin.

## Figure 3

Clinical treatment and outcome in 10 children with NDI. **A.** Simplified tonicity balance for the neonate ID#3 with NDI, excreting hypotonic urine and receiving 0.19% saline. When considering separately the balances for water and Na, the excess Na administration from 0.19% saline becomes immediately obvious. The red box in the middle represents the total body water compartment of a patient with NDI. A patient with NDI excretes hypotonic urine, detected with the urine Na concentration of 12 mmol/l. If 1 L of urine output is replaced with 1L of 0.19% saline, this will not change the fluid balance, but lead to a net gain of 20.5 mmol of Na. In the neonate of 3.5 kg with estimated 2.8L of total body water, this would lead to an increase in the plasma Na concentration of approximately  $20.5 \text{ mmol} / 2.8\text{L} = 7.3 \text{ mmol/l}$ . **B.** Serum sodium and treatment in the case #3. **C.** Follow up of the serum sodium level in ten patients with treatment of thiazides and indometacin.