Endocrine, *vol. 24, no. 1, 55–59, June 2004* 0969–711X/04/24:55–59/$25.00

Clinical Characteristics of Eight Patients with Congenital Nephrogenic Diabetes Insipidus

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Congenital nephrogenic diabetes insipidus (NDI) is characterized by the insensitivity of the distal nephron to arginine vasopressin. Clinical knowledge of this disease is based largely on case reports. For this study, we investigated the clinical findings of eight patients in terms of age at onset, age at diagnosis, main complaint, results of physical examination, the diagnosis, the effect of treatment, kidney function, and presence or absence of gene defects. The main complaints of all eight cases at initial examination were unknown fever, failure to thrive, and short stature. Polyuria and polydipsia are not always the chief complaints with congenital NDI. In one case, diabetes insipidus could be diagnosed based only on the results of a 5% hypertonic saline test. In six cases, we found abnormalities in the V2 receptor gene. Initially, trichlormethiazide therapy was shown to have a significant effect on polyuria; however, this effect decreased over time. In one patient with partial NDI, the addition of trichlormethiazide twice a day to 1-desamino-8-D-arginine vasopressin increased urine osmolality in the morning and caused nocturia to disappear. Results of ^{99m}Tc-diethylenetriamine pentaacetic acid kidney scintigraphy revealed a slight decrease in glomerular filtration rate in three patients. No patient experienced serious renal dysfunction.

Key Words: Nephrogenic diabetes insipidus; diagnosis; V2 receptor gene; treatment; kidney function.

Introduction

Congenital nephrogenic diabetes insipidus (NDI) is caused by a genetic defect in the arginine vasopressin signal transduction in the collecting duct cell; this defect results in an inability to produce a normal antidiuretic response to this hormone. In most cases, NDI is caused by a mutation in the vasopressin type 2 (V2) receptor gene, resulting in X-linked recessive inheritance *(1)*. The autosomal recessive and dominant forms of NDI, which are caused by mutations in the aquaporin-2 (AQP2) water channel gene, have a much lower prevalence *(2,3)*. The primary clinical consequence of these genetic defects is polyuria, causing dehydration and compensatory polydipsia. In infancy, however, children present with a variety of other signs. Several long-term clinical sequelae have also been reported *(4)*. In addition, urogenic complications, from slight dilatation of the urinary tract to severe hydronephrosis, have been reported *(5–8)*.

Because of the sporadic occurrence of NDI, it is difficult to determine the prevalence of the various features and complications of this disease. Moreover, reports of only the most severe cases in the literature may cause an overestimation of the complications experienced by these patients. Furthermore, in most reported studies of patients with NDI, gene analysis was not done to determine the type of mutation present.

In the present study, we describe eight male patients with NDI in whom clinical diagnosis was confirmed by gene analysis. We place special emphasis on the clinical presentation of these patients.

Results

Clinical Presentation

Age at diagnosis is shown in Table 1. Four children were diagnosed during the first year of life, and three children were diagnosed during the second year of life. As a result of the presence of affected siblings, two patients were diagnosed in the neonatal period. However, the mean age of all patients at the first examination at the hospital was 5 mo, and the mean age at diagnosis was 24 mo. One child was not diagnosed until age 11. The main complaints at the initial examination were unknown fever, failure to thrive, and short stature (Table 1). No parent of any patient mentioned the presence of polyuria or polydipsia as the primary complaint, although these symptoms were revealed in all patients by asking their parents detailed questions later.

All eight cases underwent a water restriction test (Table 2). During the water restriction test, plasma osmolarity increased to only 287 mOsm/kg in case V, which was not high enough

Received August 12, 2003; Revised February 28, 2004; Accepted April 14, 2004.

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Table 1
Clinical Presentation of Eight Patients with Congenital Nephrogenic Diabetes Insipidus[a]

			Symptoms at first examination					
Case	Age	Sex	Polyuria	Unknown fever	Failure to thrive or short stature	Presence of affected siblings	The age of the first examination	The age of the diagnosis
I-1	24	male	+	+*	–	+	3M	7M
I-2	22	male	+	–	–	+*	1M	1M
I-3	5	male	+	–	–	+*	20D	20D
II	18	male	+	+	+*	–	7D	11Y
III	16	male	+	–	+*	–	3M	3M
IV	9	male	+	+*	+	+	8M	1Y5M
V	8	male	+	+*	–	–	8D	1Y2M
VI	5	male	+	+*	–	–	2Y0M	2Y0M

[a]The mean age of the first examination at the hospital was 5 mo old and the mean age of the diagnosis was 24 mo old in all patients. No patient complained of polyuria or polydipsia at the main complaint. Asterisk = chief complaint. D, day; M, month; Y, year.

Table 2
Results of a Water Restriction Test in Eight Cases[a]

	Water restriction test		
Case	Maximum plasma osmolarity (mOsm/kg)	Maximum urine osmolarity (mOsm/kg)	Maximum plasma ADH (pg/mL)
I-1	294	73	N.D.
I-2	293	63	N.D.
I-3	312	131	38.3
II	301	90	12.0
III	320	84	N.D.
IV	300	137	142.5
V	287	198	8.9
VI	294	90	55.6

[a]In case V, diabetes insipidus was not diagnosed by this test alone.

Table 3
Results of Pitressin or DDAVP Loading Test in Eight Cases[a]

		Urinaly-c-AMP (n mol/min)	
Case	Maximum urine osmolarity (mOsm/kg)	preload	afterload
Pitressin test (0.1 U iv.)			
I-1	69	N.D.	N.D.
I-2	82	N.D.	N.D.
DDAVP test			
	Intranasally (3–20 μg) / Intravenously (0.3 μg/kg)		
I-3	N.D. / 163	0.57	0.26
II	70 / 90	1.21	1.60
III	137 / 69	0.65	1.47
IV	97 / 190	0.39	0.72
V	89 / 394	0.70	0.63
VI	87 / 124	N.D.	N.D.

[a]In case V, urine osmolarity, which did not increase enough during intranasal DDAVP loading, rose to 394 mOsm/kg during intravenous DDAVP loading.

to diagnose diabetes insipidus. In this case, the diagnosis of diabetes insipidus was made based on the results of the 5% hypertonic saline test, which showed an insufficient rise of urine osmolarity (279 mOsm/kg) when plasma osmolality level rose from 288 to 302 mOsm/kg in response to infusion of 5% hypertonic saline (data not shown). 1-Desamino-8-D-arginine vasopressin (DDAVP) test or pitressin test results are shown in Table 3. In case V, an intravenous infusion test of DDAVP indicated an increase in urine osmolality up to 394 mOsm/kg, although intranasal DDAVP loading did not increase urine osmolality. Urine osmolality for all the other cases remained under 300 mOsm/kg during intravenous DDAVP loading. In addition, cAMP was analyzed in the urine of five patients. No elevations in urine cAMP levels following DDAVP loading were seen in any of the five cases (Table 3).

Genetic Analysis

Analysis of the V2 receptor and AQP2 genes was done in seven patients (Table 4). Cases I-1, I-2, and I-3, who were from a single family, had the mutation AG to CG of splicing acceptor of the second intron of V2 receptor. This mutation has been predicted to result in aberrant splicing and in an apparently nonfunctional V2 receptor *(9)*. Case III showed microdeletion at two bases, which caused frame shift and

Table 4
Analysis of the V2 Receptor and Aquapolin-2 Genes was Performed in Seven Patients

Case	Age	V2 receptor mutation Type	V2 receptor mutation Codon change	AQP2 mutation
I-1	24	Splicing	Intron2*	(–)
I-2	22			
I-3	5			
III	16	2bp-deletion	All change after 201, Stop 253	(–)
IV	9	(–)		
V	8	Missense	R104C	(–)
VI	5	6bp-deletion	APF305-307V	(–)

*Splice acceptor AG → CG.

Table 5
Drug Treatment for Eight Patients[a]

Case	Treatment Thiazide	Helium	Spronolactone	Indomethacin	DDAVP	The effect of thiazide (L/m^2/day) Pretreatment	Just after the treatment start	Current
I-1	+	+				7.5	2.9	4.6
I-2	+	+				6.9	2.9	4.0
I-3	+		+			3.2	2.5	3.8
II	+	+				6.5	3.7	6.3
III	+	+		+⇒–		4.0	2.8	4.7
IV	+	+		+⇒–		8.3	4.2	8.8
V	+		+	+⇒–	+	9.3	8.1	2.5*
VI	+		+			8.2	4.5	5.2

[a]Initially we observed a significant effect of trichlormethiazide therapy on polyuria. However, this effect decreased over time. Asterisk (in case 5) = urine volume during combination therapy with a thiazide and DDAVP.

was thought to be a disease-causing mutation *(10)*. Case VI showed microdeletion at six bases, which lead to a predicted change of APF (codon 305–307) to V. This mutation has not been reported previously. However, the function of the deduced mutant protein is likely to be unsatisfactory due to reported mutations in the neighboring genes that cause receptor dysfunction *(11–13)*. Case V showed a C to T transition at nucleotide position +310 in exon 2. This mutation leads to a predicted change of arginine (codon 104, CGC) to cysteine (TGC). This is a missense mutation encoding within the first extracellular loop of the V2 receptor polypeptide, named R104C. Inaba et al. confirmed that the sulfhydryl group of the cysteine-104 caused most of the R104C mutation receptor dysfunction *(14)*. Case IV did not show an abnormality in either the V2 receptor or AQP2 gene; however, clinical data, including the presence of remarkable polyuria and the results of the water restriction and DDAVP loading tests, supported a diagnosis of NDI. No clear relationship was found between clinical and genetic findings.

Using the clinical and genetic data, we determined that cases I-1, I-2, I-3, II, III, IV, and VI had NDI. Case V was diagnosed with partial NDI.

Drug Treatment

All patients were treated with a trichlormethiazide and a low-salt diet from diagnosis until the present. Initially, trichlormethiazide therapy was shown to significantly decrease polyuria. However, this effect decreased over time (Table 5). We tried indomethacin therapy in three patients, but all three suffered from hypothermia or headache and treatment was stopped because of these side effects.

For case V, a partial NDI patient, the addition of trichlormethiazide twice a day helped DDAVP act more effectively on the partially responding V2 receptors. The effect was to increase urine osmolality in the morning and cause nocturia to disappear, as described previously *(15)*. Thus, combination therapy with a thiazide and DDAVP was useful for this patient, who showed a partial vasopressin response.

Table 6
DTPA Kidney Scintigraphy Results
in Four Examples of Patients Older than 15 yr[a]

		DTPA GFR (mL/min)	
Case	Age	Light	Right
I-1	24	55.5	49.0
I-2	22	40.0	47.0
II	18	70.1	47.9
III	16	66.0	67.0

[a]The decline in kidney function in these patients was not clinically significant, although they experienced excessive drinking and polyuria.

Urological Problems

All patients underwent echo and intravenous pyelogram examinations to determine the presence of expansion of the urinary tract. In case III, expansion of the bladder was seen starting at age 5. No other patient showed expansion of the urinary tract before age 8 (data not shown).

We also examined the ^{99m}Tc-diethylenetriamine pentaacetic acid (DTPA) kidney scintigraphy results in four examples of patients older than 15 yr to examine the glomerular filtration rate (GFR) levels. We found slight decrease in GFR levels in three patients (40–49 mL/min). However, we did not consider these findings to represent a significant decline in kidney function, despite the presence of excessive drinking and polyuria (Table 6).

Discussion

NDI is established in patients as the cause of severe hypotonic polyuria based on a deficient antidiuretic response to endogenous and exogenous arginine vasopressin. Polyuria and polydipsia are not always the chief complaints associated with congenital NDI, although they are essential for its diagnosis. Without the presence of affected siblings, it can take time to determine the diagnosis of NDI. However, when symptoms such as remarkable dehydration and poor weight gain are noted, the diagnosis of NDI must be done quickly, as brain damage may be caused by high plasma osmolality. In our investigation, the primary complaints of all patients did not include polyuria or polydipsia. However, based on the chief complaints of growth disorder, poor weight gain, and short stature, we considered the diagnosis of NDI.

The water restriction test is important for the diagnosis of NDI. If an effective water limit is not determined, a definitive diagnosis cannot be made. In addition, this test cannot diagnose partial NDI, as occurred in case V. On the other hand, a 5% hypertonic saline test can be used to ensure the diagnosis, even for patients with partial NDI, such as our case V.

In case V, urine osmolarity, which did not increase enough during intranasal DDAVP loading, rose to 394 mOsm/kg during intravenous DDAVP loading. We diagnosed this case as partial NDI based on clinical features and gene analysis. In addition, combination therapy with DDAVP and a thiazide may be useful in patients with partial NDI *(15)*. Intranasal DDAVP loading is generally an effective way to diagnose NDI; however, as shown in case V, intravenous loading was needed to make a diagnosis of partial NDI. Based on the findings of intravenous DDAVP loading, an effective treatment became possible. Thus, it may be significant to use intravenous DDAVP loading instead of intranasal DDAVP loading for some patients.

Most (>90%) congenital NDI patients have mutations in the AVPR2 gene, the Xq28 gene coding for the vasopressin V2 receptor. In <10% of the families studied, congenital NDI has an autosomal recessive inheritance with mutations of AQP2 gene, that is, the vasopressin-sensitive water channel, were identified. In our study, we found abnormalities in the V2 receptor gene in six cases. None of these patients experienced an increase in cAMP levels in the urine after DDAVP loading, as has been shown in conventional reports.

There is no known effective therapy for NDI. Although thiazides have been shown to have an effect early in treatment, this effect appears to diminish over time. In most of cases, significant polyuria occurs even if thiazide treatment is continued. In case V, urine osmolarity rose subnormally after the DDAVP test even with an intravenous DDAVP load, because the effect of thiazide was insufficient. However, combination therapy can cause nocturia to disappear *(15)*, which can have a positive impact on quality of life.

There are few long-term data about renal function and NDI. None of our cases experienced serious renal dysfunction. In fact, kidney function did not decrease even in case III, in whom bladder expansion was noted at age 5.

In conclusion, polyuria and polydipsia are not always the chief complaints with congenital NDI. We could diagnose one case of NDI only by using a 5% hypertonic saline loading. It may be significant that the DDAVP test was performed intravenously in the patient who was diagnosed with partial NDI. The effect of thiazides gradually decreased over time, and urine volume increased again in some patients. DDAVP coupled with thiazide therapy may be useful for some patients with NDI who have shown a partial response to DDAVP. There were no cases of serious renal dysfunction in our series.

Materials and Methods

We performed an analysis of clinical data from eight male NDI patients (aged 5 to 24 yr) from five Japanese families, collected between 1995 and 2002 in our hospital. All patients were diagnosed by the presence of polyuria and polydipsia, a water restriction and a DDAVP or pitressin loading test. For the DDAVP load examination, intravenous infusion of 0.3 μg/kg had been compared with the results of intranasal loading in six of the eight patients. In addition, we

evaluated cAMP levels in the urine before and after DDAVP loading.

In seven patients, analysis of the V2 receptor and AQP2 genes was performed as described previously *(16,17)*. Genomic DNA was prepared from peripheral leukocytes by standard methods. About V2 receptor gene, the gene was amplified by PCR with oligonucleotide primers (forward: 5'-TG ACCATCCCCTCTCAATCTTC-3'; reverse; 5'-TCCCTC TTTCCTGCACTCCT-3'). A total of 12 difference primers (sense and antisense) were needed for direct sequencing of the PCR product as described previously *(17)*. For the AQP2 gene, the first PCR was performed using oligonucleotide primers: 5'-AGTGCGAGAGCGAGTGCC-3' and 5'-TCACAAGCGTCCGTCGG-3'. Then, the nested PCR was performed with the first PCR product. Primer sequence were: sense primer exon1: 5'-TCCTGGCCCTGAGACAGCTGG-3' and antisense primer exon1: 5'-CCCAGGACCTGCCC CTTGT-3'; sense primer exon2: 5'-CAGGAAGATGGAG CCAGAGAG-3' and antisense primer exon2: 5'-GTCCCT CTTGGGGTCTCTGTG-3'; sense primer exon3: 5'-CTTC CTGTTCTGTCCT-3' and antisense primer exon3: 5'-CCC CATCCCATGCTATTCCAG-3'; sense primer exon4: 5'-G TTGCCAAACCGCCCTCTCCGT-3' and antisense primer exon4: 5'-TCACAAGCGTCCGTCGG-3'. Direct sequence of the PCR product was performed using the specific primers described above with an autoanalyzer (ABI PRISM 377 DNA Sequencer; Applied Biosystems).

In terms of treatment, we examined the effect of the therapy that was selected for each patient on urine volume.

In addition, we used the DTPA kidney scintigraphy to evaluate kidney function in some patients.

We investigated the clinical findings of these eight cases in terms of age at onset, age at diagnosis, main complaint, results of physical examination, the diagnosis, the effect of treatment, kidney function, and presence or absence of gene defects.

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