

Identification of two novel *aquaporin-2* mutations in a Thai girl with congenital nephrogenic diabetes insipidus

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Received: 11 April 2008 / Accepted: 1 May 2008 / Published online: 13 May 2008
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Abstract *Objective* To describe a Thai girl with congenital nephrogenic diabetes insipidus (NDI) and perform mutation analysis of the *AQP2* gene. *Design:* Case report. *Patient* A 6-year old girl with a history of failure to thrive, polydipsia and polyuria was studied. Polyuria and polydipsia were observed within the first few months of life. Despite normal serum osmolality and electrolyte, the result of water deprivation test was compatible with a diagnosis of NDI. *Methods* The entire coding regions of the *AQP2* gene were assessed by polymerase chain reaction and sequencing analysis. The presence of mutations was also confirmed by restriction enzyme digestion analysis. *Results* Two heterozygous novel missense mutations were identified. Both were located in exon 1; a guanine-to-thymine substitution at nucleotide position 3 (c.3G→T) inherited from her mother and a guanine-to-adenine at position 85 (c.85G→A) inherited from her father, resulting in a methionine to isoleucine at codon 1 (p.M1I) and glycine to serine at codon 29 (p.G29S), respectively. These mutations have never been previously described and were not detected in 100 ethnic-matched unaffected control chromosomes. *Conclusion* We report two novel mutations of the *AQP2* gene, p.M1I and p.G29S, associated with autosomal recessive congenital NDI. This study expands the genotypic spectrum of *AQP2* mutations

and emphasizes an important role of genetic testing for definite diagnosis and genetic counseling.

Keywords Congenital nephrogenic diabetes insipidus · *aquaporin-2* (*AQP2*) · Novel mutations · Polyuria

Introduction

Nephrogenic diabetes insipidus (NDI) is characterized by an inability of renal collecting ducts to concentrate urine in response to arginine vasopressin (AVP). It can be either acquired or inherited. Congenital NDI is a rare inherited disorder. Its clinical manifestations include vomiting, anorexia, failure to thrive, fever, and constipation which usually appear within the first year of life [1]. About 90% of cases are inherited in an X-linked recessive pattern (OMIM 304800). This form is caused by mutations in the *arginine vasopressin receptor* (*AVPR2*) gene [2]. Not more than 10% of cases are due to mutations in the *aquaporin-2* (*AQP2*) gene [3], with the majority being inherited in an autosomal recessive manner (OMIM 125800). A much rare autosomal dominant form has also been reported [4].

At least 40 different mutations in the *AQP2* gene causing NDI have been described (<http://www.hgmd.cf.ac.uk>, Accessed March 2008). Of these, most are missense mutations, which are responsible for recessive NDI. The autosomal dominant form of NDI caused by the same gene has been diagnosed in only seven families [5–9]. Most of the identified mutations are short nucleotide deletions. All *AQP2* mutations in dominant NDI are located in the C-terminus, whereas those causing autosomal recessive NDI are scattered throughout the gene [4].

In this study, we described the clinical features of a Thai girl with congenital NDI and performed mutation analysis

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of all the coding regions of the *AQP2* gene. Two novel heterozygous missense mutations were identified.

Case report

A 6-year old girl was referred to our clinic at the age of 3 years with a history of failure to thrive and polyuria. Prenatal history was unremarkable. Her birth weight was 3,200 g. She was the only child of healthy, non-consanguineous parents. There was no family history of NDI. She developed polyuria and polydipsia within the first few months of life. At the age of 6 months, she started to have poor weight gain. Physical examination at 3 years old revealed that her weight and height were below the third percentile with head circumference of 48 cm (25th percentile). Initial laboratory evaluation revealed serum sodium of 136 mmol/l, serum potassium of 3.7 mmol/l, total serum calcium of 9.1 mg/dl, blood urea nitrogen of 8 mg/dl, and creatinine of 0.5 mg/dl. Serum and urine osmolalities were 287 mosm/kg H₂O and 104 mosm/kg H₂O, respectively. She then underwent a water deprivation test. The results were compatible with a diagnosis of NDI with elevated serum osmolality (290 mosm/kg H₂O) despite a low urinary osmolality (32 mosm/kg H₂O) and no increase in urine osmolality after nasal 1-desamino-8-D-arginine vasopressin (DDAVP) administration (Table 1). Brain magnetic resonance imaging showed no abnormalities. Abdominal ultrasonography demonstrated normal kidneys and urinary tract. The patient was treated with hydrochlorothiazide (2 mg/kg/day) and indomethacin (2 mg/kg/day). Her polyuria subsequently improved. The 24-h urine volumes were substantially decreased from 4.2–4.7 l/m²/day prior to treatment to 1.9–2.6 l/m²/day

thereafter. However, the patient still had nocturnal enuresis. During a 3-year follow up, no clinical dehydration was observed. Her serum creatinine and electrolytes had been within a normal range. On her last visit at 6 years of age, her weight was 15 kg (10th percentile) while her height was 103.5 cm (3rd percentile). Due to her persistent short stature, serum IGF-1 levels and thyroid function tests were evaluated which revealed a slightly decreased levels of IGF1 (73.39 ng/ml, normal 78–310 ng/ml), but normal free T4 and TSH levels. A clonidine stimulation test resulted in a growth hormone peak of 12.7 ng/ml (normal > 7.5 ng/ml). Her neurodevelopmental status remained appropriate for age.

Materials and methods

Molecular methods

After informed consent was obtained, genomic DNA was extracted from peripheral leukocytes according to standard protocols. Since the patient was a female with unaffected parents and negative family history, an autosomal recessive NDI was considered. The *AQP2* gene analysis was then performed. All four exons of the *AQP2* gene were amplified by polymerase chain reaction (PCR) using sets of primers and reaction conditions as shown in Table 2. We used 100 ng of genomic DNA, 1 × PCR buffer (Promega, Madison, WI), 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.2 μM of each primer, and 0.5 U Taq DNA polymerase (Promega) in a total volume of 20 μl. The PCR products were verified for correct size on ethidium bromide-stained 1.5% agarose gel. The PCR products were then treated with ExoSAP-IT (USP Corporation, Cleveland, OH) according to the

Table 1 The results of water deprivation test

Time (h)	Urine output (ml/h)	Urine sp.gr.	Serum sodium (mmol/l)	Serum osm (mosm/kg)	Urine osm (mosm/kg)	BW (kg)
0	130	1.000	137	274	25	10.56
1	179	1.000				10.18
2	125	1.000	142	290	32	10.03
DDAVP nasal solution 2.5 μg IN						
3	127	1.000				9.97
4	125	1.001				
DDAVP 0.14 μg SC						
5	172	1.000				
6	204	1.000				
7	192	1.000				
8	207	1.000	138	274	31	10.43

Note: sp. gr—specific gravity; Osm—osmolality; BW—body weight; DDAVP—1-desamino-8-D-arginine vasopressin; IN—intranasal; SC—subcutaneous

Table 2 Oligonucleotides and PCR conditions for *AQP2* mutation analysis

Exon	Primer Sequences for PCR 5' to 3'		Annealing Temperature (°C)
	Forward	Reverse	
1	GAGCGATAGAGTGCGAGAGC	GGATGGGCTCTGGGTGATGT	55
2,3	GACTGCAGGTGGACAGGAAG	GCTGGAATAGCATGGGATGG	55
4	AGCAGCTGGCGTTGTCGTTG	GAGGCTGTGAGCAGCTAGTG	61

manufacturer's recommendations, and sent for direct sequencing at the Macrogen Inc., Seoul, Korea. The sequence was analyzed using Sequencher (version 4.2; Gene Codes Corporation, Ann Arbor, MI). For two novel missense mutations, restriction enzyme digestion was used to confirm their presence in the patient and parents as well as to screen in 100 control chromosomes from unaffected ethnic-matched individuals.

Protein sequence comparisons

AQP2 orthologs were first identified through a BLAST search of the non-redundant database using *Homo sapiens* AQP2, accession NP_000477, as the reference sequence. All known and complete AQP2 sequences were included from vertebrate and invertebrate lineages. These files in FASTA format were then analyzed by ClustalX 1.81 program. The human AQP2 was aligned with rhesus monkey (*Macaca mulatta*; XP_001110572), cow (*Bos taurus*; NP_001094669), dog (*Canis familiaris*; XP_543678), rat (*Rattus norvegicus*; NP_037041), mouse (*Mus musculus*; NP_444418.1), and fly (*Drosophila melanogaster*; NP_523697). The program classified amino acids by the variation in polarity, assessing both amino acid class conservation and evolutionary conservation at any given site.

Results

Analysis of the *AQP2* gene by PCR-sequencing of the proband revealed two novel heterozygous missense mutations, located in exon 1; a guanine-to-thymine substitution at nucleotide position 3 (c.3G→T) and a guanine-to-adenine at position 85 (c.85G→A), resulting in a methionine to isoleucine substitution at codon 1 (p.M1I) and a glycine to serine substitution at codon 29 (p.G29S), respectively (Fig. 1). These mutations have never been previously described and were not detected in 100 ethnic-matched unaffected control chromosomes (data not shown). Restriction enzyme digestion analysis of exon 1 of parental genomic DNA revealed that the mother was heterozygous for the c.3G→T mutation, while the father was heterozygous for the c.85G→A mutation (Fig. 2).

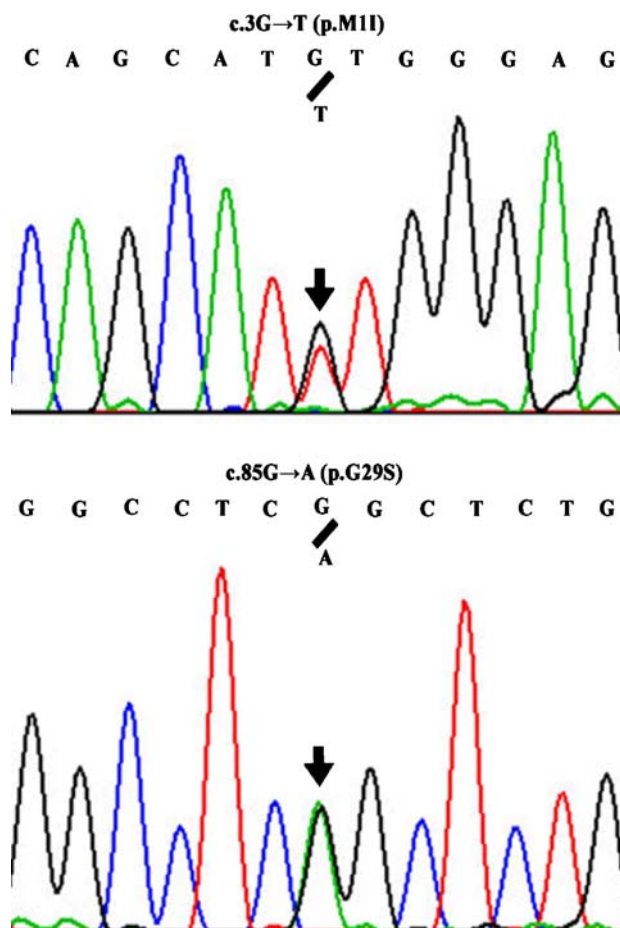


Fig 1 Mutation analysis. Upper and lower panels are electropherograms of the patient heterozygous for c.3G→T (p.M1I) and c.85G→A (p.G29S) mutations, respectively. Each mutation is indicated by an arrow

Discussion

We described a Thai girl with a rare form of congenital NDI caused by compound heterozygous missense mutations in the *AQP2* gene. Both mutations in exon 1 have not been previously described. One was a single base transversion, c.3G→T, resulting in a methionine to isoleucine substitution at codon 1 (p.M1I). The other was a single base transition, c.85G→A, resulting in a glycine to serine substitution at codon 29 (p.G29S). The c.3G→T was

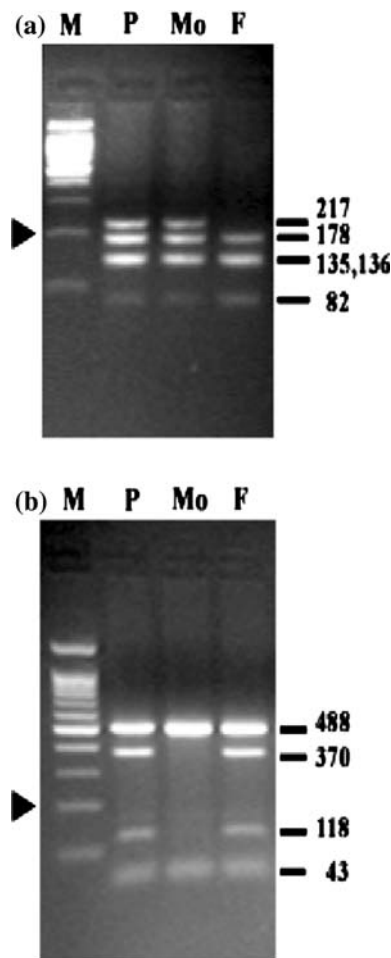


Fig. 2 Restriction enzyme digestion analysis. M: 100-bp marker. P: patient. Mo: mother of the patient. F: father of the patient. The 200-bp band is indicated by an arrow head. **(a)** Restriction enzyme analysis of the PCR products showing the c.3G→T lacking the cleavage site for the restriction endonuclease *Nla*III resulting in an uncut 217-bp band. The wild type alleles are digested resulting in 178, 136, 135, and 82-bp bands. **(b)** Restriction enzyme analysis of the PCR products showing the c.85G→A creating additional cleavage site for the restriction endonuclease *Dde*I resulting in 370, 118-bp products. The wild type alleles are digested resulting in 488 and 43-bp bands

transmitted maternally and the c.85G→A was transmitted paternally, confirming an autosomal recessive inheritance.

The *AQP2* gene encodes a vasopressin-regulated water channel which has a significant role in reabsorption of water in the renal collecting duct and consequently in

concentrating urine. AQP2 is predominantly expressed in the principal cells of the collecting duct and selectively permeable to water molecules. It is found to be translocated between the apical membrane and subapical storage vesicles in response to vasopressin [10]. Functional studies of several *AQP2* missense mutants causing autosomal recessive NDI have shown that the majority are misfolded and thus retained in the endoplasmic reticulum (ER). However, some causing disrupted channels have been demonstrated [11].

The start codon mutation, p.M1I, may lead to abolition of the translation start site. This type of mutation has been detected in several genetic disorders, e.g., cystic fibrosis, congenital adrenal hyperplasia, mucopolysaccharidosis type 1, and ornithine transcarbamylase [12–15]. It remains possible that an alternative in-frame initiation codon may be used. However, using the putative downstream alternative initiation codon at position 46 (p.M46), the second ATG located in exon 1 would result in elimination of the entire first and a small part of the second transmembrane domains of the protein. Mutations located in the first transmembrane domain leading to improper function of the AQP2 protein have been reported [16, 17]. Further in vitro analysis will clarify its molecular mechanism leading to congenital NDI.

The p.G29S mutation is located in the first transmembrane domain of AQP2. As this segment forms a part of the AQP2 water pores, mutations in this part might cause misfolding of the protein and thus inability to translocate to the plasma membrane. The p.G29S mutation was next to the one that has been previously described in one family with autosomal recessive NDI, the p.L28P. Functional characterization of this mutant was performed in *Xenopus* oocytes and revealed that it conferred reduced water permeability to oocytes as a result of ER retention [17]. Due to its close proximity to the p.L28P, the pathogenicity of the p.G29S mutation could be explained by the similar mechanism. Even though no in vitro study was performed to investigate the functional consequence of the p.G29S, there are several lines of evidence supporting it as a disease-causing mutation. First, the glycine at codon 29 is located at a highly conserved transmembrane domain (Fig. 3). Second, this variant has not been reported to be a

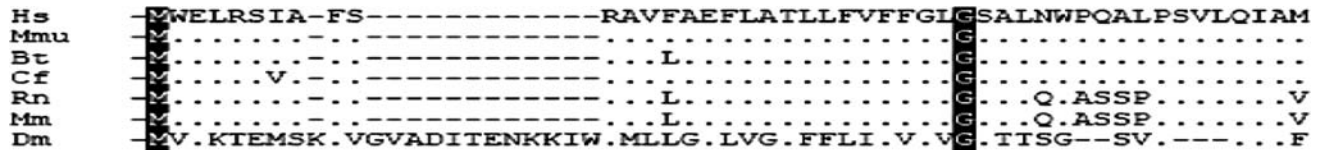


Fig. 3 Sequence alignment of the AQP2. The sites of all amino acid variants found in this study are shaded in all conserved species. Sites that are 100% conserved across all sequences are indicated by dots (.). Hs—*Homo sapiens*; Mmu—*Macaca mulatta*; Bt—*Bos Taurus*; Cf, *Canis familiaris*; Rn—*Rattus norvegicus*; Mm—*Mus musculus*; Dm—*Drosophila melanogaster*

polymorphism in NCBI SNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>), Ensembl (<http://www.ensembl.org/index.html>) or PupaSUITE/PupaSNP (<http://pupasuite.bioinfo.cipf.es/>) databases. And lastly, it was not detected in 100 ethnic-matched control chromosomes.

Growth retardation is among the common features found in congenital NDI. However, it has not been studied systematically. In this study, we demonstrated that the patient had slightly low IGF-1 levels with normal growth hormone secretion. Insufficient energy and protein intake can cause reduced IGF-I levels [18, 19]. Therefore, it is possible that failure of normal growth is partly due to reduced intake of solid food because of very high fluid ingestion.

In summary, we reported a Thai girl with autosomal recessive NDI caused by two novel missense mutations in the *AQP2* gene, the c.3G→T mutation in one allele and the c.85G→A in the other allele, which were inherited from her mother and father, respectively. This study expands the genotypic spectrum of *AQP2* mutations and emphasizes an important role of genetic testing for definite diagnosis, early initiation of appropriate therapy and genetic counseling.

Acknowledgments We would like to thank the patient and her family for participation in this study. We are grateful to Dr. Pairoch Chotivitayatarakorn for patient referral and Dr. Nuanphong Rienmanee for her excellent care of the patient. This study was supported by the Development Grants for New Faculty/Researchers, the Research Unit Grant from Chulalongkorn University, and the Thailand Research Fund.

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