CASE REPORT

# Novel compound aquaporin 2 mutations in nephrogenic diabetes insipidus

Raphael D. Liberatore Junior,[I] Juliana G. Carneiro,[II] Franciele B. Leidenz,[II] Rachel Melilo-Carolino,[II] Helena C. Sarubi,[II] Luiz De Marco[II]

[I] Faculdade de Medicina de São José do Rio Preto, Department of Pediatrics, São José do Rio /SP, Brazil. [II] Universidade Federal de Minas Gerais, Faculdade de Medicina, Department of Surgery, Belo Horizonte/MG, Brazil.

Email: ldemarco@ufmg.br
Tel.: 55 31 3409-9134

## INTRODUCTION

Nephrogenic diabetes insipidus (NDI) is a rare disease that is characterized by the excretion of abnormally large volumes of urine, due to the inability of the kidneys to concentrate urine in response to arginine vasopressin (AVP). Classical NDI symptoms include polydipsia and polyuria in infants during the first year of life. Acquired NDI is the most common form of this disease in adults. The majority of inherited cases are caused by mutations in the arginine vasopressin V2 receptor (AVPR2) gene (MIM# 300538) on chromosome Xq28, which leads to functional defects in the AVPR2. The aquaporin (AQP2) gene (MIM# 107777) on chromosome 12q13 (1) is associated with the disease (2,3) in the minority of cases (~10%). The present study identified two novel compound heterozygous mutations in the AQP2 gene, H201Y and G211R, in one female patient with congenital NDI.

## CASE REPORT

A breastfeeding two-month-old girl was admitted to the emergency room after 12 days of fever of unknown origin and weight loss. The patient had received 20 μg of 1-desamino[8-D-arginine]vasopressin (dDAVP) intranasally, but this intervention did not decrease her urine output. Physical examination revealed a severely dehydrated and highly irritable infant with no other clinical abnormalities. The infant's height-for-age ratio was in the 3$^{rd}$ percentile, and her initial laboratory profile revealed hypernatremia (172 mEq/ml) and a low urine density (1.005). The patient's basal sodium was within the normal range after an increase in her fluid intake and a low sodium diet, and she was subjected to laboratory investigation to ascertain the diagnosis of NDI according to established criteria (4). A short water deprivation test was performed under a strictly controlled medical setting, but this test was stopped six hours later because of weight loss (3%). Her urinary osmolality was 263 mOsmol/kg at six hours and increased to 300 mOsmol/kg one hour after dDAVP administration

(20 μg intranasally). The patient was allowed water after the administration of dDAVP. The T1-weighted images from a brain MRI did not reveal the hyperintense signal that is normally emitted by the posterior pituitary gland (i.e., there was no "bright spot"). The child's parents were also subjected to diagnostic procedures to exclude nephrogenic diabetes insipidus. The patient was the second child of a healthy young and non-consanguineous couple with no diabetes insipidus symptoms. The patient was a postfertilization baby for whom the female sex had been chosen, and her older brother had hemophilia. The family pedigree is shown in Figure 1a. Hydrochlorothiazide (5 mg/kg/twice daily) and amiloride (20 mg/m$^2$/day) were initiated, but only a partial response was observed; namely, the patient's basal urine osmolality increased to 456 mOsmol/kg two weeks after the initiation of these two medications. A remission of symptoms occurred when indomethacin (1.0 mg/kg/day) was subsequently added. Two weeks after initial administration of this medication, the patient's urine osmolality rose to 587 mOsmol/kg. The patient's height-for-age ratio was in the 50$^{th}$ percentile at age four, while she was under careful surveillance and receiving medications.

The parents signed an informed consent that was approved by the Institutional Ethics Committee in accordance with the ethical standards of the 1964 Declaration of Helsinki.

## MATERIALS AND METHODS

Whole blood cells were collected from the patient and her parents for molecular analyses. Genomic DNA was isolated using the Wizard Genome DNA Purification Kit® (Promega, Madison, WI) according to the manufacturer's instructions. All coding and flanking regions of all exons of the AVPR2 gene were amplified by PCR using previously described sets of primers (5). The AQP2 gene oligonucleotides included the following sequences: exon 1, forward CA-TCCTGGCCCTGAGACA, reverse TACAAGGGATTCCC-CAGGAC; exon 2, forward GACAGGAAGATGGA-GCCAGA, reverse TGGAGTGGTCTGTGTGTCTGT; exon 3, forward ACAAGGACTTCCTGCCCTGT, reverse TCCC-ATGCTATTCCAGCTCT; and exon 4, forward TAATGTC-GGGGAGGAGAGGT, reverse CACGTCCAGGAAGCAG-CTA. Briefly, PCR was performed in a final volume of 25 μl containing IIB Buffer 10x, 0.2 mM dNTP, 10 pmol of each primer, 0.625 U Taq polymerase and 60 ng/μl DNA. PCR products were purified using the GFX PCR DNA and Gel

Compound aquaporin 2 mutations in diabetes insipidus
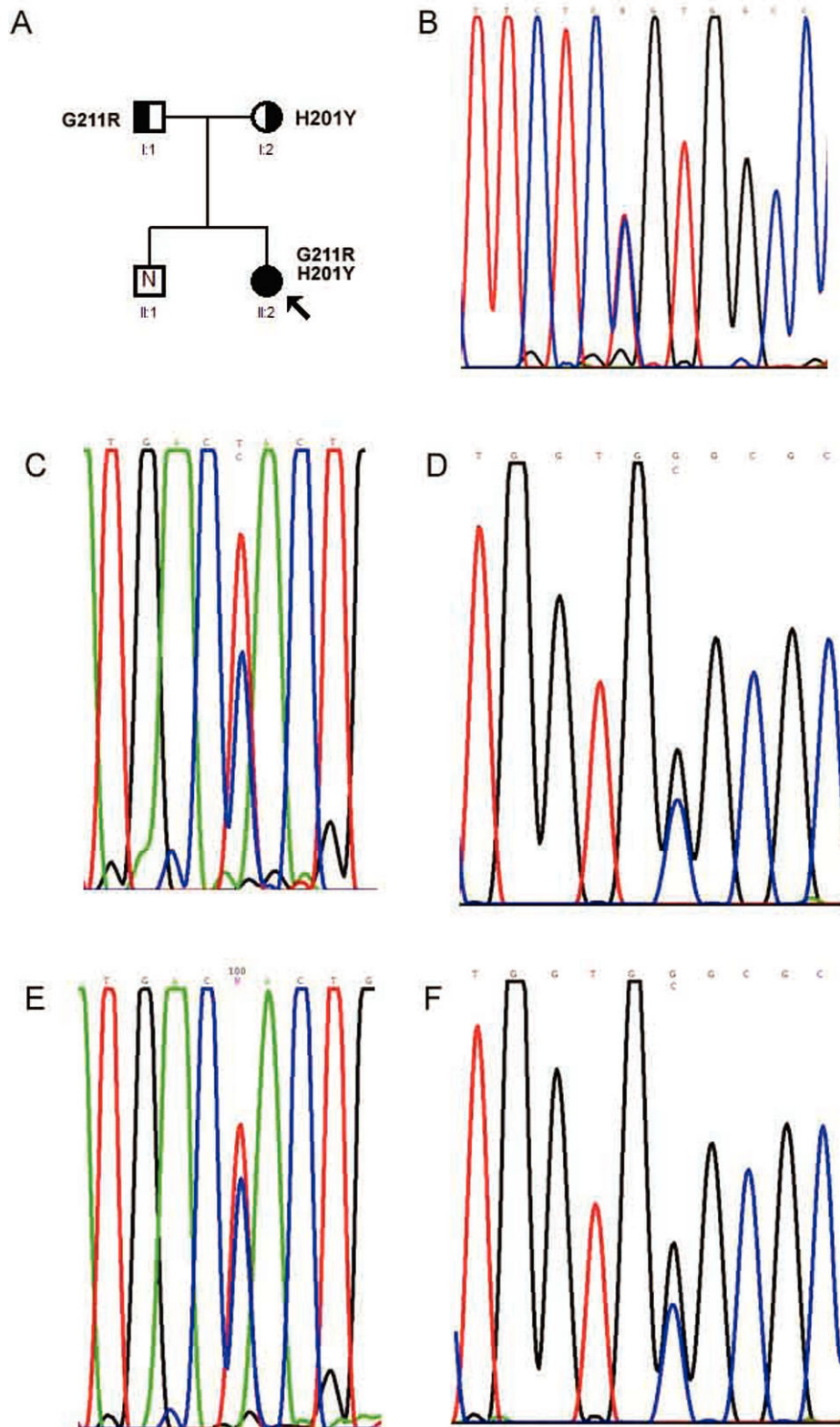Junior RDL et al.

CLINICS 2012;67(1):79-82



**Figure 1 -** (a) Pedigree of the Brazilian family with NDI; the proband is indicated by an arrow. An open square with an inset (N) indicates that the individual was unaffected. (b) c.491T>C polymorphism; (c and e) heterozygosis at c.601C>T (H201Y) of the proband and her mother; and (d and f) heterozygosis at c.697C>G (G211R) of the proband and her father.

Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer's instructions and sequenced in an ABI Prism® 3130 Genetic Analyzer using the ABI Prism Dye Terminator sequencing kit (Applied Biosystems, Foster City, CA) according to the standard protocol. Sequences were performed in the sense and

CLINICS 2012;67(1):79-82

Compound aquaporin 2 mutations in diabetes insipidus
Junior RDL et al.

antisense directions in duplicate using separate DNA extractions.

## RESULTS

A diagnosis of NDI due to mutations of the *AVPR2* gene was excluded based on the patient's sex, although certain female carriers have a partial response to dDAVP due to skewed X-chromosome inactivation (6), and also based on the direct sequencing of the entire coding and exon-flanking gene regions, which demonstrated a wild-type sequence. Sequencing analyses of all four *AQP2* exons revealed that the patient was a compound heterozygote with two novel point mutations in exons 3 and 4. One point mutation was a C-to-T transversion at position 601 (c.601C>T) in exon 3 (H201Y) (Figure 1c), which was inherited from her mother (Figure 1e), and a C to G transition at position 697 (c.697C>G) in exon 4 (G211R) (Figure 1d), which was inherited from her father (Figure 1f). A previously described polymorphism at position 491 (c.491T>C; S167S) was also present (Figure 1b). Neither parent exhibited any clinical or biochemical signs of diabetes insipidus.

## DISCUSSION

This report described two novel missense mutations in a heterozygote female infant with inherited NDI. Severe polyuria and polydipsia began soon after birth, and these findings in association with the child's sex and the failure of dDAVP to relieve symptoms suggested that NDI was caused by mutation(s) of the *AQP2* gene. A full mutation analysis of the AVP receptor gene demonstrated no germ-line mutations. NDI that is caused by mutations in the *AQP2* gene are inherited as either an autosomal recessive or a dominant trait (7,8). The sequencing analyses of the *AQP2* gene in our patient revealed a compound heterozygosity that was inherited from both parents. Heterozygote mutation carriers are not affected. Therefore, no clinically important phenotype was expected or observed in the patient's parents. Interestingly, the patient's height-for-age ratio at age four was within the normal range. Patients with mutations in the *AQP2* gene have a short (9) or normal stature (10,11). The response to therapy in this child was notably better than the response of other patients with autosomal-recessive NDI due to *AQP2* gene mutations. The reasons for this improved response are not known, but the presence of a compound heterozygote mutation may underlie this unusually good response.

The human *AQP2* gene is located on chromosome 12q13. This gene has four exons and three introns, and it is predicted to code a 271-amino-acid protein. AQP2 is a single polypeptide chain with six transmembrane domains, which is similar to other aquaporins, and both terminal ends are located inside the cell (3). The first intracellular and the third extracellular loops contain the asparagine-proline-alanine (NPA) motif that is conserved in all members of the membrane integral protein (MIP) family. This motif may play a role in the formation of functional water-selective pores, but it is no longer thought to confer water selectivity (12). In addition, the phosphorylation of serine at position 256 by PKA in the cytoplasmic COOH-terminus of AQP2 is essential for its distribution from intracellular vesicles to the apical plasma membrane (13,14).

To date, 66 distinct *AQP2* gene mutations have been described, and the vast majority (86%) of these mutations are associated with an autosomal recessive mode of transmission (MIM #125800). Several compound mutations within this gene have also been described (6,9,11,15-19). Most mutations in patients with autosomal-recessive NDI are localized between the first and the last transmembrane domains. This segment forms the AQP2 water pore, and the mutation-induced misfolding illustrates the sensitivity of the pore to structural changes (14). A compound recessive mutation has been described previously in a female patient in which one of the mutations was located in the conserved region of the last transmembrane domain of AQP2, and this mutation resulted in a misfolded protein (6).

The mutations in our analysis, H201Y and G211R, were located on the extracellular and transmembrane domains, respectively, and these domains are probably critical for protein function. Both histidine 201 and glycine 211 are highly conserved amino acids between species. The wild-type glycine, which is the smallest amino acid, is located next to proline, which is responsible for protein folding. The mutations resulting from a substitution of tyrosine (uncharged polar amino acid) for histidine and arginine for glycine severely alter AQP2 structure and disrupt water absorption.

In conclusion, this study described a compound hetero-zygosity that was characterized by two novel mutations in *AQP2* exons 3 and 4 in an infant female patient. These combined mutations probably caused a disruption in the protein, but functional studies are necessary to understand the effects of these mutations on AQP2.

## AUTHOR CONTRIBUTIONS

Liberator Jr. RD was responsible for thepatient care. Carneiro JG, Leidenz FB, Melilo-Carolino R, Sarubi HC were responsible for the experimental work. De Marco L was responsible for the experimental design and manuscript writing.

## REFERENCES

1. Sasaki S, Fushimi K, Saito H, Saito F, Uchida S, Ishibashi K, et al. Cloning, characterization, and chromosomal mapping of human aqua-porin of collecting duct. J Clin Invest. 1994;93:1250-6, doi: 10.1172/JCI117079.
2. Deen PMT, Verdijk MAJ, Knoers NVAM, Wieringa B, Monnens LAH, van Os CH, et al. Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. Science. 1994;264:92-4, doi: 10.1126/science.8140421.
3. Fujiwara TM, Bichet DG. Molecular biology of hereditary diabetes insipidus. J Am Soc Nephrol. 2005;16:2836-46, doi: 10.1681/ASN.2005040371.
4. Sands JM, Bichet DG. Nephrogenic diabetes insipidus. Ann Intern Med. 2006;144:186-94.
5. Boson WL, Della Manna T, Damiani D, Miranda DM, Gadelha MR, Liberman B, et al. Novel vasopressin type 2 (AVPR2) gene mutations in Brazilian nephrogenic diabetes insipidus patients. Genet Test. 2006;10:157-62, doi: 10.1089/gte.2006.10.157.
6. Fujiwara TM, Bichet DG. Molecular biology of hereditary diabetes insipidus. J Am Soc Nephrol. 2005;16:2836-46, doi: 10.1681/ASN.2005040371.
7. van Lieburg AF, Verdijk MAJ, Knoers VVAM, van Essen AJ, Proesmans W, Mallmann R, et al. Patients with autosomal nephrogenic diabetes insipidus homozygous for mutations in the aquaporin 2 water-channel gene. Am J Hum Genet. 1994;55:648-52.
8. Mulders SM, Bichet DG, Rijss JPL, Kamsteeg E-J, Arthus M-F, Lonergan M, et al. An aquaporin-2 water channel mutant which causes autosomal

Compound aquaporin 2 mutations in diabetes insipidus
Junior RDL et al.

CLINICS 2012;67(1):79-82

dominant nephrogenic diabetes insipidus is retained in the Golgi complex. J Clin Invest. 1998;102:57-66, doi: 10.1172/JCI2605.

9. Marr N, Bichet DG, Hoefs S, Savelkoul PJ, Konings IB, De Mattia F, et al. Cell-biologic and functional analyses of five new aquaporin-2 missense mutations that cause recessive nephrogenic diabetes insipidus. J Am Soc Nephrol. 2002;13:2267–77, doi: 10.1097/01.ASN.0000027355.41663.14.

10. Moon S-S, Kim H-J, Choi Y-K, Seo H-A, Jeon J-H, Lee J-E, et al. Novel mutation of *aquaporin-2* gene in a patient with congenital nephrogenic diabetes insipidus. Endocr J. 2009;56:905-10, doi: 10.1507/endocrj.K09E-078.

11. Tsutsumi Z, Inokuchi T, Tamada D, Moriwaki Y, Ka T, Takahashi S, et al. Compound heterozygous mutation of aquaporin 2 gene in woman patient with congenital nephrogenic diabetes insipidus. Intern Med. 2009;48:437-40, doi: 10.2169/internalmedicine.48.1642.

12. Beitz E, Wu B, Holm LM, Schultz JE, Zeuthen T. Point mutations in the aromatic/arginine region in aquaporin 1 allow passage of urea, glycerol, ammonia, and protons. Proc Natl Acad Sci U S A. 2006;103:269-74, doi: 10.1073/pnas.0507225103.

13. Kuwahara M, Asai T, Terada Y, Sasaki S. The C-terminal tail of aquaporin-2 determines apical trafficking. Kidney Int. 2005;68:1999-2009, doi: 10.1111/j.1523-1755.2005.00654.x.

14. Robben JH, Knoers NV, Deen PM. Cell biological aspects of the vasopressin type-2 receptor and aquaporin 2 water channel in nephrogenic diabetes insipidus. Am J Physiol Renal Physiol. 2006;291:F257-F270, doi: 10.1152/ajprenal.00491.2005.

15. Tajima T, Okuhara K, Satoh K, Nakae J, Fujieda K. Two novel aquaporin-2 mutations in a sporadic Japanese patient with autosomal recessive nephrogenic diabetes insipidus. Endocr J. 2003;50:473-6, doi: 10.1507/endocrj.50.473.

16. Cheong HI, Cho SJ, Zheng SH, Cho HY, Ha IS, Chou Y. Two novel mutations in the aquaporin 2 gene in a girl with congenital nephrogenic diabetes insipidus. J Korean Med Sci. 2005;20:1076-8, doi: 10.3346/jkms.2005.20.6.1076.

17. Iolascon A, Aglio V, Tamma G, D'Apolito M, Addabbo F, Procino G, et al. Characterization of two novel missense mutations in the AQP2 gene causing nephrogenic diabetes insipidus. Nephron Physiol. 2007;105:33-41, doi: 10.1159/000098136.

18. Sahakitrungruang T, Tee MK, Rattanachartnarong N, Shotelersuk V, Suphapeetiporn K, Miller WL. Functional characterization of vasopressin receptor 2 mutations causing partial and complete congenital nephrogenic diabetes insipidus in Thai families. Horm Res Paediatr. 2010;73:349-54, doi: 10.1159/000308167.

19. Leduc-Nadeau A, Lussier Y, Arthus MF, Lonergan M, Martinez-Aguayo A, Riveira-Munoz E, et al. New autosomal recessive mutations in aquaporin-2 causing nephrogenic diabetes insipidus through deficient targeting display normal expression in Xenopus oocytes. J Physiol. 2010;588:2205-18, doi: 10.1113/jphysiol.2010.187674.