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A Molecular Genetic Study of X-Linked Nephrogenic Diabetes Inspidus†

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= Abstract = **X-linked nephrogenic diabetes insipidus (NDI) is a rare disease characterized by absent vasopressin V2 receptor responses. Recently, the vasopressin V2 receptor gene (*AVPR2* gene) was cloned, and several mutations have been reported in association with NDI. We analyzed the *AVPR2* gene in a family with X-linked NDI. Genomic DNA was isolated from peripheral blood samples from 11 members of the family. Four overlapping segments covering the entire coding sequence of the *AVPR2* gene were amplified by polymerase chain reaction (PCR) with the genomic DNA. And DNA sequencing was done after subcloning of the PCR products into a plasmid vector. We found a mutation in the *AVPR2* gene, common in 4 symptomatic male patients in the family. It was a novel missense point mutation at the codon 219 CTG to CCG resulting a transition of leucine-219, located in the 5th transmembrane domain of the receptor molecule, to proline. The T to C transition generated a new recognition site for a restriction enzyme *Sma* I. And 3 heterozygous female carriers of the mutant gene were detected by the pattern of *Sma* I digestion of PCR products including the mutation site. These results provided a strong evidence for that the mutation was the cause of NDI in this family.**

Key Words: *Nephrogenic diabetes insipidus, Vasopressin, Vasopressin V2 receptor, Vasopressin V2 receptor gene, Mutation*

INTRODUCTION

Congenital nephrogenic diabetes insipidus (NDI) is a rare hereditary disorder in which renal response to arginine vasopressin is impaired. As a consequence, it is characterized by hyposmotic polyuria despite high plasma concentrations of vasopressin. The action of vasopressin is mediated by 2 sets of receptors, V1 and V2 receptors, and the latter is responsible for the antidiuretic effect (Reeves and Andreoli 1989). In most affected families, NDI is transmitted as an X-linked recessive disorder (McKusick 1990). Linkage studies have located the responsible gene on the long arm of the X chromosome in region 28 (Xq28) (Knoers *et al.* 1987; Kambouris *et al.* 1987; Knoers *et al.* 1988). And the gene for the vasopressin V2 receptor (*AVPR2* gene) was cloned and also localized to Xq28 (Lolait *et al.* 1992), strongly suggesting that the receptor may be the site of the defect in NDI. Soon after, this suggestion was confirmed by several papers reporting mutations in the *AVPR2* gene associated with NDI (Rosenthal

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et al. 1992; Ouweland *et al.* 1992; Pan *et al.* 1992; Holtzman *et al.* 1993a; Merendino *et al.* 1993; Bichet *et al.* 1993a; Bichet *et al.* 1993b; Holtzman *et al.* 1993b; Yokoyama *et al.* 1993). Recently, we diagnosed a 1-year-old boy as NDI on the basis of clinical symptoms and the lack of increase of urine osmolality after administration of 1-desamino-8-D-arginine vasopressin. There were 3 more male patients with clinically proven NDI among his family members. We analyzed the *AVPR2* gene in the family and found a novel point mutation in the gene.

MATERIALS AND METHODS

Genomic DNA was isolated from peripheral blood samples obtained from 11 members of the family including 4 symptomatic males according to standard methods (Sambrook *et al.* 1989). Four oligonucleotide primers were used to amplify overlapping segments of the entire coding sequence of the *AVPR2* gene by polymerase chain reaction (PCR) with the genomic DNA (Table 1 and Fig. 1). For each DNA samples, 3 combinations of primers were used; primers 1 and 4, primers 2 and 3, and primers 3 and 4. Each reaction mixture contained 0.5-1.0ug of genomic DNA, 100pmol of each primer, 20nmol of each deoxynucleotide triphosphate, 2.5U Taq DNA polymerase and standard buffer solution. Initial denaturation was done at 95℃ for 5 min followed by 30 cycles of amplification at the following temperatures; 95℃ for 1 min, 58℃ for 2 min and 72℃ for 2 min. Final extension was done at 72℃ for 7 min. The 3 different sized PCR products from each male patients were purified and subcloned into *Eco* RV-cut pBluescript KS (Stratagene Inc. , La Jolla, CA). And double-stranded DNA sequencing was done using the dideoxy chain termination method (Sanger *et al.* 1977) with the Sequenase kit, version 2.0 (United States Biochemical Corp., Cleveland, OH, USA). The entire length of the coding sequence of *AVPR2* gene was checked and compared to the reported sequence of human *AVPR2* gene (Birnbaumer *et al.* 1992). The mutant sequence was confirmed by sequencing at least 2 additional independent subclones.

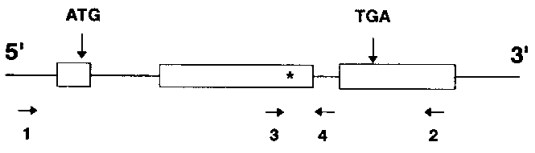

Fig. 1. The structure of the V2 receptor gene and the position of 4 PCR primers(1-4) in the gene. The open squares are exons and solid lines are introns. (ATG, start codon; TGA, stop codon; * site of the mutation in this report)

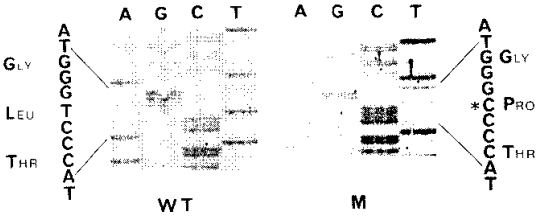

Fig. 2. The DNA sequences in an unaffected family member (wild-type, WT) and the proband (mutant, M). The DNA and protein sequences are read from bottom to top, and the asterisk denotes the position of the point mutation.

Table 1. Primers used for PCR

Primer No.	Sequence	Orientation	Corresponding *AVPR2* gene sequence
1	5'-CCAGGACTGGCCATACTG-3'	sense	−177 to −160
2	5'-CCAGCTCAGTGAGCTGAC-3'	antisense	+1,812 to +1,829
3	5'-AACGTGGAAGGTGGCAGC-3'	sense	+905 to +922
4	5'-AGCCCTAGCCACGGATACA-3'	antisense	+1,277 to +1,295

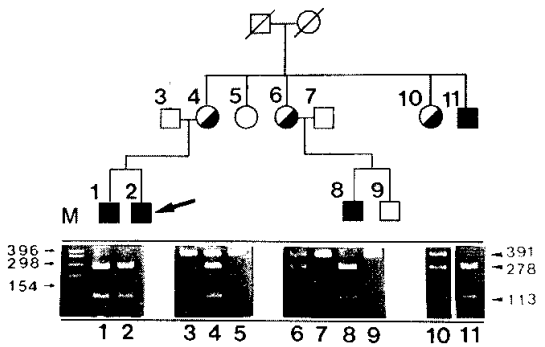

Fig. 3. Pedigree of the family and the results of polyacrylamide gel electrophoresis of the 391-bp PCR products after *Sma* I digestion. The amplified DNA of unaffected members (open circles and squares) remained intact showing a single 391-bp band on the gel, and those of 4 affected males (homozygous for the mutation, solid squares) were cut into 2 pieces (278- and 113-bp bands). The 3 female carriers(heterozygous for the mutation, half-solid circles) had a mixture of normal and mutant fragments showing 3 bands. (M, molecular size markers; thick arrow, the proband)

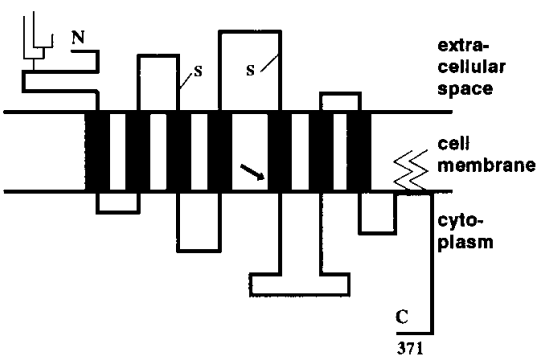

Fig. 4. The structure of V2 receptor protein (371 amino acids) and the site of the mutation in the molecule (the arrow). (N, amino-terminal; C, carboxy-terminal)

RESULTS

We found a mutation in the *AVPR2* gene, common in 4 symptomatic male patients by sequencing the entire coding sequences of the gene using 3 different, overlapping subclones. It was a missense point mutation, located between the primers 3 and 4 (Fig. 1), changing codon 219 CTG to CCG and resulting in transition of leucine-219 to proline (Fig. 2). The T→C transition generated a new recognition site for a restriction endonuclease *Sma* I (5'-CCCGGG3'). So, we digested the 391-base pair (bp) PCR fragments (using primers 3 and 4) of 11 family members with *Sma* I, and separated the fragments by 10% polyacrylamide gel electrophoresis to segregate the normal and mutant sequences (Fig. 3). The mutant fragments of 4 male patients were cut into 2 pieces, 113- and 278-bp fragents, with the enzyme, while normal fragments remained intact. Three female carriers had a mixture of normal and mutant fragments indicating heterozygosity for the mutation. Additionally, to rule out the possibility of genetic polymorphism, the 391-bp DNA fragment was amplified by PCR from the genomic DNAs of 18 unrelated normal volunteers. And all of the fragments remained intact after digestion with *Sma* I enzyme. The codon of leucine-219 is located in the 5th transmembrane domain of the V2 receptor protein molecule (Fig. 4).

DISCUSSION

To date, more than 20 mutations in the *AVPR2* gene have been reported in association with congenital NDI (Rosenthal *et al.* 1992; Ouweland *et al.* 1992; Pan *et al.* 1992; Holtzman *et al.* 1993a; Merendino *et al.* 1993; Bichet *et al.* 1993a; Bichet *et al.* 1993b; Holtzman *et al.* 1993b; Yokoyama *et al.* 1993). These mutations include insertions or deletions with or without frame shifts, nonsense mutations, and missense mutations. And the defects are clustered in the 3rd transmembrane domain, the 3rd extracellular domain, and the 3rd cytoplasmic domain of the 7-membrane spanning V2 receptor molecule. These 3 domains are known to play an important role in ligand binding and effector coupling

(Savarese and Fraser 1992; Ostrowski *et al.* 1992).

The mutation reported here lies not within these functionally important domains, but within the 5th transmembrane domain of the molecule. And we can not tell how the single amino acid transition (leucine to proline) in this domain causes functional impairment of the receptor molecule because of the lack of functional studies. However, we found an assortment of the mutation in 4 affected males, and observed both mutant and normal alleles in their mothers, obligate carriers. These findings strongly suggest that the mutation is the cause of NDI in this family. And proline, an imino acid, is very rigid and known to creates fixed kink in a polypeptide chain (Darnell *et al.* 1990). So, the substituting proline can result in a misfolding of the protein and a functional defect.

Most of the mutations in the *AVPR2* gene reported to date have been based only on documenting assortment of the defects in affected individuals, but not on functional studies like a in vitro expression of the defective protein (Rosenthal *et al.* 1993). However, the functional studies are essential to clarify how specific mutations result in defective ligand binding or signal transduction. Congenital NDI is the 1st disease associated with defective G protein-coupled hormone receptors. The functional studies of the naturally occuring mutations will provide much information about the whole family of the G protein-coupled hormone receptors and probably lead to new therapies. In addition, identification of a specific mutation in a family has clinical importance by enabling to detect carrier women and diagnose their offsprings early.

REFERENCES

Bichet DG, Arthus M-F, Lonergan M, Hendy GN, Paradis AJ, Fujiwara TM, Morgan K, Gregory MC, Rosenthal W, Didwania A, Antaramian A, Birnbaumer M. X-linked nephrogenic diabetes insipidus mutations in North America and the Hopewell hypothesis. J Clin Invest 1993; 92: 1262-8a

Bichet DG, Birnbaumer M, Arthus M-F, Lonergan M, Hendy GN, Rosenthal W, Goodyer P, Fujiwara M, Morgan K. Identification of eleven new V_2 receptor mutations in families with X-linked nephrogenic diabetes insipidus. (abst) J Am Soc Nephrol 1993; 4:260b

Birnbaumer M, Seibold A, Gilbert S, Ishido M, Barberis C, Antaramian A, Brabet P, Rosenthal W. Molecular cloning of the receptor for human antidiuretic hormone. Nature 1992; 357:333-5

Darnell J, Lodish H, Baltimore D(eds). Molecular cell biology 2nd edn. Scientific American Books, Inc., New York, 1990, p45

Holtzman EJ, Harris HW Jr, Kolakowski LF Jr, Guay-Woodford LM, Botelho B, Ausiello DA. Brief report: a molecular defect in the vasopressin V2-receptor gene causing nephrogenic diabetes insipidus. N Engl J Med 1993; 328:1534-7 a

Holtzman EJ, Kolakowski LF, Geifman-Holtzman O, O'Brien DG, Rasoulpour M, Guillot AP, Harris HW, Ausiello DA. Molecular defects in the vasopressin V2 receptor(*AVPR2*) gene causing nephrogenic diabetes insipidus (CNDI). (abst) J Am Soc Nephrol 1993; 4:815 b

Kambouris M, Dlouhy SR, Trofatter JA, Conneally PM, Hodes ME. Localization of the gene for X-linked nephrogenic diabetes insipidus to Xq28. Am J Med Genet 1988; 29:239-46

Knoers N, Heyden H van der, Oost BA van, Ropers HH, Monnens L, Willems J. Nephrogenic diabetes insipidus: close linkage with markers from the distal long arm of the human X-chromosome. Hum Genet 1988; 80:31-8

Knoers N, Heyden H van der, Oost BA van, Monnens L, Willems J, Ropers HH. Tight linkage between nephrogenic diabetes insipidus and DXS52. Cytogenet Cell Genet 1987; 46:640

Lolait SJ, O'Carroll A-M, McBride OW, Konig M, Morel A, Brownstein MJ. Cloning and characterization of a vasopressin V2 receptor and possible link to nephrogenic diabetes insipidus. Nature 1992; 357:336-9

McKusick VA. Mendelian inheritance in man 9th

edn. The Johns Hopkins University Press, Baltimore, 1990, p1585

Merendino JJ Jr, Spiegel AM, Crawford JD, O'Carroll A-M, Brownstein MJ, Lolait SJ. Brief report: a mutation in the V2-receptor gene in a kindred with X-linked nephrogenic diabetes insipidus. N Engl J Med 1993; 328:1538-41

Ostrowski J, Kjelsberg MA, Caron MG, Lefkowitz RJ. Mutagenesis of the β_2-adrenergic receptor: how structure elucidates function. Annu Rev Pharmacol Toxicol 1992; 32:167-83

Ouweland AMW van den, KnoopMT, Knoers NVAM, Markslag PWB, Rocchi M, Warren ST, Ropers HH, Fahrenholz F, Monnens LAH, Oost BA van. Mutations in the vasopressin type 2 receptor gene(*AVPR2*) associated with X-linked nephrogenic diabetes insipidus. Nature Genet 1992; 2:99-102

Pan Y, Metzenberg A, Das S, Jing B, Gitschier J. Mutations in the V2 vasopressin receptor gene are associated with X-linked nephrogenic diabetes insipidus. Nature Genet 1992; 2:103-6

Reeves WB, Andreoli TE. Nephrogenic diabetes insipidus. In: Schriver CR, Beaudet AL, Sly WS, Valle D(eds) The metabolic basis of inherited diseases 6th edn. McGraw-Hill, New York, 1989, pp1985-2011

Rosenthal W, Antaramian A, Gilbert S, Birnbaumer M. Nephrogenic diabetes insipidus. A V2 vasopressin receptor unable to stimulate adenylyl cyclase. J Biol Chem 1993; 268:13030-3

Rosenthal W, Seibold A, Antaramian A, Lonergan M, Arthus MF, Hendy GN, Birnbaumer M, Bichet DG. Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. Nature(Lond.) 1992; 359:233-5

Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual 2nd edn. Cold Spring Harbor Labor atory Press, New York, 1989, pp9. 17-9

Sanger F, Nicklen S, Coulson AB. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci USA 1977; 74:5463-7

Savarese TM, Fraser CM. In vitro mutagenesis and the search for structure-function relationships among G protein-coupled receptors. Biochem J 1992; 283:1-19

Yokoyama K, Yamauchi A, Itoh T, Izumi M, Takama T, Fujiwara Y. A mutation in the vasopressin type 2 receptor(V2R) gene in a patient with hereditary nephrogenic diabetes insipidus(HNDI). (abst) J Am Soc Nephrol 1993; 4:828