Am. J. Hum. Genet. 55:278–286, 1994

Nature and Recurrence of AVPR2 Mutations in X-linked Nephrogenic Diabetes Insipidus

Daniel G. Bichet,[1] Mariel Birnbaumer,[7] Michèle Lonergan,[1] Marie-Françoise Arthus,[1] Walter Rosenthal,[7] Paul Goodyer,[2] Hubert Nivet,[8] Stéphane Benoit,[8] Philip Giampietro,[9] Simonetta Simonetti,[10] Alfred Fish,[11] Chester B. Whitley,[12] Philippe Jaeger,[13], Joseph Gertner,[9] Maria New,[9] Francis J. DiBona,[14] Bernard S. Kaplan,[15] Gary L. Robertson,[16] Geoffrey N. Hendy,[3,4] T. Mary Fujiwara,[2,3,5] and Kenneth Morgan[3,5,6]

[1] Unité de recherche clinique, Centre de recherche et Service de néphrologie, Département de Médecine, Hôpital du Sacré-Coeur de Montréal, Université de Montréal, and Departments of [2]Pediatrics, [3]Medicine, [4]Physiology, [5]Human Genetics, and [6]Epidemiology and Biostatistics, McGill University, Montreal; [7]Department of Cell Biology, Baylor College of Medicine, Houston; [8]Unité de néphrologie pédiatrique, Centre hospitalier universitaire de Tours, Tours; [9]Department of Pediatrics, The New York Hospital—Cornell Medical Center, New York; [10]Divisione Mallatie Metaboliche e Genetiche, Ospedale "Giovanni XXIII," Bari, Italy; [11]Department of Pediatric Nephrology and [12]Department of Pediatrics and Institute of Human Genetics, University of Minnesota Hospitals, Minneapolis; [13]Policlinique médicale universitaire, Hôpital de l'Ile, Berne; [14]Aiken Renal Physicians, Aiken, SC; [15]Division of Nephrology, The Children's Hospital of Philadelphia and Department of Pediatrics, The University of Pennsylvania, Philadelphia; and [16]Clinical Research Center and Northwestern University Medical School, Chicago

Summary

X-linked nephrogenic diabetes insipidus (NDI) is a rare disease with defective renal and extrarenal arginine-vasopressin V_2 receptor responses due to mutations in the AVPR2 gene in Xq28. We analyzed 31 independent NDI families to determine the nature and recurrence of AVPR2 mutations. Twenty-one new putative disease-causing mutations were identified: 113delCT, 253del35, 255del9, 274insG, V88M, R106C, 402delCT, C112R, Y124X, S126F, W164S, S167L, 684delTA, 804insG, W284X, A285P, W293X, R337X, and three large deletions or gene rearrangements. Five other mutations—R113W, Y128S, R137H, R181C, and R202C—that previously had been reported in other families were detected. There was evidence for recurrent mutation for four mutations (R113W, R137H, S167L, and R337X). Eight de novo mutation events were detected (274insG, R106C, Y128S, 167L [twice], R202C, 684delTA, and R337X). The origins were maternal (one), grandmaternal (one), and grandpaternal (six). In the 31 NDI families and 6 families previously reported by us, there is evidence both for mutation hot spots for nucleotide substitutions and for small deletions and insertions. More than half (58%) of the nucleotide substitutions in 26 families could be a consequence of 5-methylcytosine deamination at a CpG dinucleotide. Most of the small deletions and insertions could be attributed to slipped mispairing during DNA replication.

Received February 10, 1994; accepted for publication April 18, 1994.

Address for correspondence and reprints: Dr. Daniel G. Bichet, Centre de recherche, Hôpital du Sacré-Coeur de Montréal, 5400, boulevard Gouin ouest, Montréal, Québec H4J 1C5, Canada.

© 1994 by The American Society of Human Genetics. All rights reserved.

0002-9297/94/5502-0007$02.00

Introduction

X-linked nephrogenic diabetes insipidus (NDI; MIM 304800 [McKusick 1990]) is generally a rare disease in which the affected male patients do not concentrate their urine after the administration of the antidiuretic hormone arginine-vasopressin (AVP) (Reeves and Andreoli 1989; Bichet 1992). The cDNA and genomic sequences encoding the human V_2 receptor have been isolated and characterized (Birnbaumer et al. 1992; Seibold et al. 1992). The sequence of the cDNA predicts a polypeptide of 371 amino acids that has seven transmembrane, four extracellular, and four cytoplasmic domains (Birnbaumer et al. 1992). The gene contains three exons and two small introns (Seibold et al. 1992). Eighteen mutations segregating with the NDI phenotype have been reported (Pan et al. 1992; Rosenthal et al. 1992; van den Ouweland et al. 1992; Bichet et al. 1993; Holtzman et al. 1993*a*, 1993*b*; Merendino et al. 1993*a*, 1993*b*; Tsukaguchi et al. 1993). One of the mutations (R137H) has been expressed in cultured mammalian cells; the mutant receptor exhibited a normal binding affinity for vasopressin, compared with the normal receptor, but failed to stimulate the G_s/adenylyl cyclase system (Rosenthal et al. 1993). In the present communication we report the identification of 21 additional AVPR2 mutations considered to cause NDI, which were found in a study of 31 independent NDI families. We combine this information in the present study with our previous studies, to discuss the nature, recurrence, and mechanisms of mutagenesis of 30 AVPR2 mutations.

Subjects and Methods

NDI Families

The 31 families are from diverse ethnic groups (table 1). The nomenclature used in previous reports (Bichet et al.

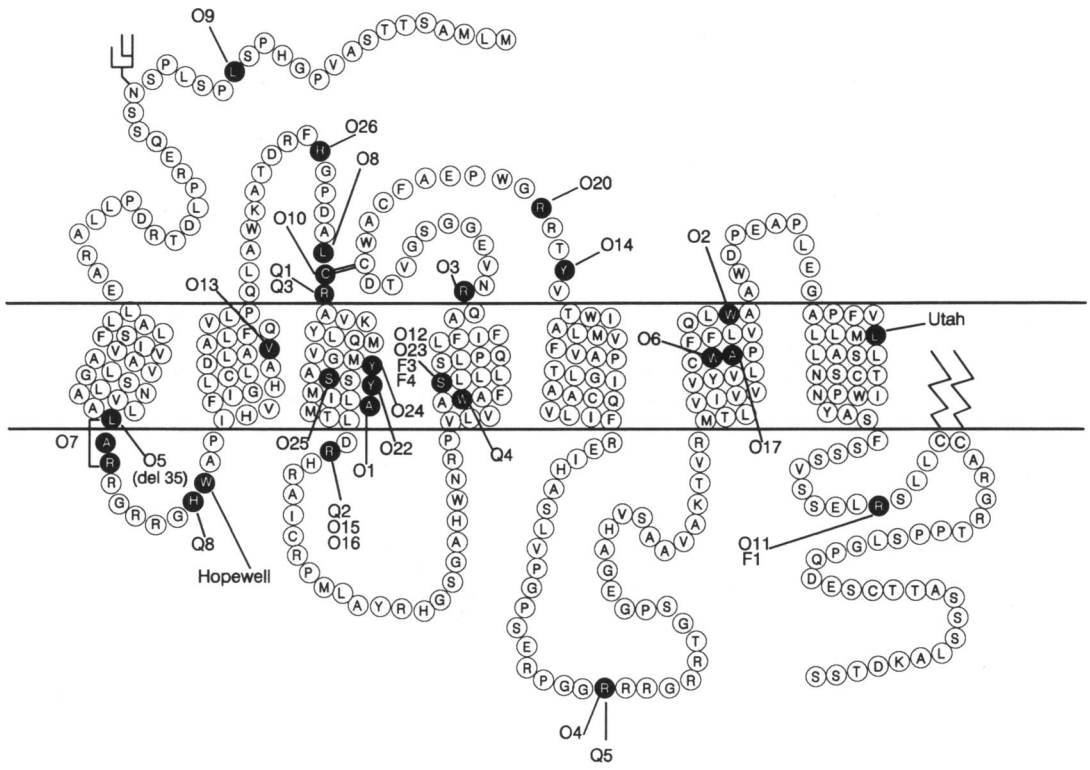

Figure 1 Schematic representation of the V_2 receptor and identification of 27 AVPR2 mutations in 37 unrelated families (three large deletions are not included). Six mutations described elsewhere by us (Rosenthal et al. 1992; Bichet et al. 1993) are included. Families are designated by "Q," "O," and "F" denominations (see text and table 1). The mutations are distributed throughout the entire V_2 receptor gene sequence.

1992, 1993; Rosenthal et al. 1992) is continued here (i.e., "Q" for families from Quebec, "F" for families from France, and "O" for other families). The pedigrees and Xq28 haplotypes for families Q_1, Q_4, and O_2–O_6 have been described elsewhere (Bichet et al. 1992). We have information on the disease segregating for at least three generations in seven families. The segregation of NDI in all the pedigrees was compatible with X-linked inheritance. All affected male patients had a documented lifelong history of polyuria and polydipsia, normal or elevated plasma concentrations of arginine vasopressin, and unconcentrated urine despite administration of arginine vasopressin and/or dDAVP (Bichet et al. 1988).

Analysis of Xq28 Haplotypes and AVPR2 DNA Sequencing

Analysis of the St14 VNTR and DNA sequencing were done as described elsewhere (Bichet et al. 1992, 1993; Rosenthal et al. 1992). In addition to the Xq28 polymorphisms St14 (DXS52) and DX13 (DXS15), used in previous studies (Bichet et al. 1992, 1993), we analyzed an *Eco*RI polymorphism 20 kb downstream of G6PD and a *Hin*dIII polymorphism within F8C (Filosa et al. 1993). Intragenic AVPR2 sequence variants were detected by direct sequencing. Together, these markers encompass a 5-Mb region that also includes the AVPR2 gene (Schlessinger et al. 1993).

For each family (except the three families with large deletions), the entire AVPR2 gene of at least one affected male and, where possible, at least one obligate carrier and one unaffected brother of a patient was sequenced. In each family, the mutation was confirmed by sequencing the region containing the mutation in all other affected males and obligate carriers. In the three families with large deletions, no fragment was obtained after amplification using our nested primers, described elsewhere (Bichet et al. 1993). We used different primers (sequences available from D.G.B.) covering the entire AVPR2 gene to characterize each large deletion.

Restriction-Enzyme Analysis of AVPR2 Mutations

The Sequence Analysis Software Package (Genetics Computer Group) was used to identify the abolition or creation of restriction sites by the mutations. Analysis of restriction-enzyme–digestion products was done by using electrophoresis on 1% agarose gels. Restriction enzymes were purchased from New England BioLabs.

Results

NDI Mutations

Twelve missense mutations (V88M, R106C, C112R, R113W, S126F, Y128S, R137H, W164S, S167L, R181C,

Table 1

AVPR2 Mutations in 37 Independent Families with X-linked NDI

Location in V2R Protein Structure	Name	Type of Mutation	Nucleotide Change	Predicted Amino Acid Change	Family and Ethnicity	Restriction-Enzyme Analysis	Comments
Mutations causing NDI:							
E_I	T7S or T/S7	Missense	A→T at 90	Thr→Ser at 7	O_8, African from Congo		NDI allele with three mutations
E_I	113delCT	Frameshift	Deletion of CT at 113–114	Frameshift 3′ to codon 16; codon 190→stop	O_9, Italian		Loss of one of two TC repeats
TM_I	253del35	Frameshift	Deletion of 35 bp (253–287)	Frameshift 3′ to codon 62; codon 190→stop	O_5, French	*Nae*I site abolished	
TM_I and C_I	255del9	In-frame deletion	Deletion of 9 bp (255–263)	Deletion of Leu62-Ala63-Arg64	O_7, Vietnamese	*Sma*I site created	
C_I	274insG	Frameshift	Insertion of G in region 274–277	Frameshift 3′ to codon 70; codon 190→stop	Q_8, French Canadian	*Sfi*I site abolished	String of four Gs
C_I	W71X	Nonsense	G→A at 284	Trp→Stop at 71	Hopewell	*Bsi* HKAI site created	Bichet et al. 1993
TM_{II}	V88M	Missense	G→A at 333	Val→Met at 88	O_{13}, North American Caucasian	*Nco*I site created	CG→CA
E_{II}	R106C	Missense	C→T at 387	Arg→Cys at 106	O_{26}, North American Caucasian		CG→TG
E_{II}	402delCT	Frameshift	Deletion of CT at 402–403	Frameshift 3′ to codon 111; codon 190→stop	O_8, African from Congo	*Ear*I site created	NDI allele with three mutations
E_{II}	C112R	Missense	T→C at 405	Cys→Arg at 112	O_{10}, North American Caucasian	*Hga*I site created	
E_{II}	R113W	Missense	C→T at 408	Arg→Trp at 113	Q_1 and Q_3, French Canadian		CG→TG; Bichet et al. 1993
TM_{III}	Y124X	Nonsense	T→G at 443	Tyr→stop at 124	O_{24}, North American Caucasian		
TM_{III}	S126F	Missense	C→T at 448	Ser→Phe at 126	O_{25}, North American Caucasian		
TM_{III}	Y128S	Missense	A→C at 454	Tyr→Ser at 128	O_{22}, North American Caucasian (Irish ancestry)		Pan et al. 1992
TM_{III}	A132D	Missense	C→A at 466	Ala→Asp at 132	O_1, Iranian		Rosenthal et al. 1992
C_{II}	R137H	Missense	G→A at 481	Arg→His at 137	Q_2, O_{15}, and O_{16}, North American Caucasian	*Msl*I site created	CG→CA; Bichet et al. 1993
C_{II}	A147V or A/V147	Missense	C→T at 511	Ala→Val at 147	O_8, African from Congo		CG→TG; NDI allele with three mutations
TM_{IV}	W164S	Missense	G→C at 562	Trp→Ser at 164	Q_4, French Canadian		
TM_{IV}	S167L	Missense	C→T at 571	Ser→Leu at 167	O_{12}, North American Caucasian O_{23}, North American Caucasian		CG→TG

					F_3 and F_4, French		
E_{III}	R181C	Missense	C→T at 612	Arg→Cys at 181	O_3, French	*Hha*I site abolished	CG→TG; Pan et al. 1992
E_{III}	R202C	Missense	C→T at 675	Arg→Cys at 202	O_{20}, Italian		CG→TG; van den Ouweland et al. 1992
E_{III}	684delTA	Frameshift	Deletion of TA at 684–685	Frameshift 3′ to codon 205; codon 257→stop	O_{14}, Swiss		
C_{III}	804insG	Frameshift	Insertion of G in region 804–809	Frameshift 3′ to codon 247; codon 258→stop	O_4, British		String of six Gs
C_{III}	804delG	Frameshift	Deletion of G in region 804–809	Frameshift 3′ to codon 247; codon 270→stop	Q_5, French Canadian		Rosenthal et al. 1992
TM_{VI}	W284X	Nonsense	G→A at 922	Trp→stop at 284	O_6, African American		
TM_{VI}	A285P	Missense	G→C at 924	Ala→Pro at 285	O_{17}, Italian	*Msc*I site created	
TM_{VI}	W293X	Nonsense	G→A at 949	Trp→stop at 293	O_2, Puerto Rican	*Sfc*I site created	
TM_{VII}	L312X	Nonsense	T→A at 1006	Leu→stop at 312	Utah		Bichet et al. 1993
C_{IV}	R337X	Nonsense	C→T at 1080	Arg→stop at 337	O_{11}, North American Caucasian		CG→TG
					F_1, French		
		Large deletion	Deletion 3′ to 181		Q_6, French Canadian		
		Large deletion	Deletion 3′ to 720		F_2, French		
		Large deletion	Deletion 3′ to 921		O_{29}, North American Caucasian		
Sequence variants not causing NDI:							
E_1	G/A176	Silent substitution	G or A at 176	No change (Leu) at 35	O_6, Arican American		
C_1	R/W64	Missense	C or T at 261	Arg→Trp at 64	O_8, African from Congo		CG→TG
C_{II}	C/G518	Silent substitution	C or G at 518	No change (Arg) at 149	O_{24}, North American Caucasian		
TM_{VII}	998A/G	Silent substitution	A or G at 998	No change (Leu) at 309	O_6–O_8, O_{10}, O_{11}, O_{13}, O_{25}, and O_{26}		
C_{IV}	C/T1064	Silent substitution	C or T at 1064	No change (Ser) at 331	O_6, African American		CG→TG
...	1187+52del12		Deletion of 12 bp	No change; deletion after stop codon	O_6, African American		

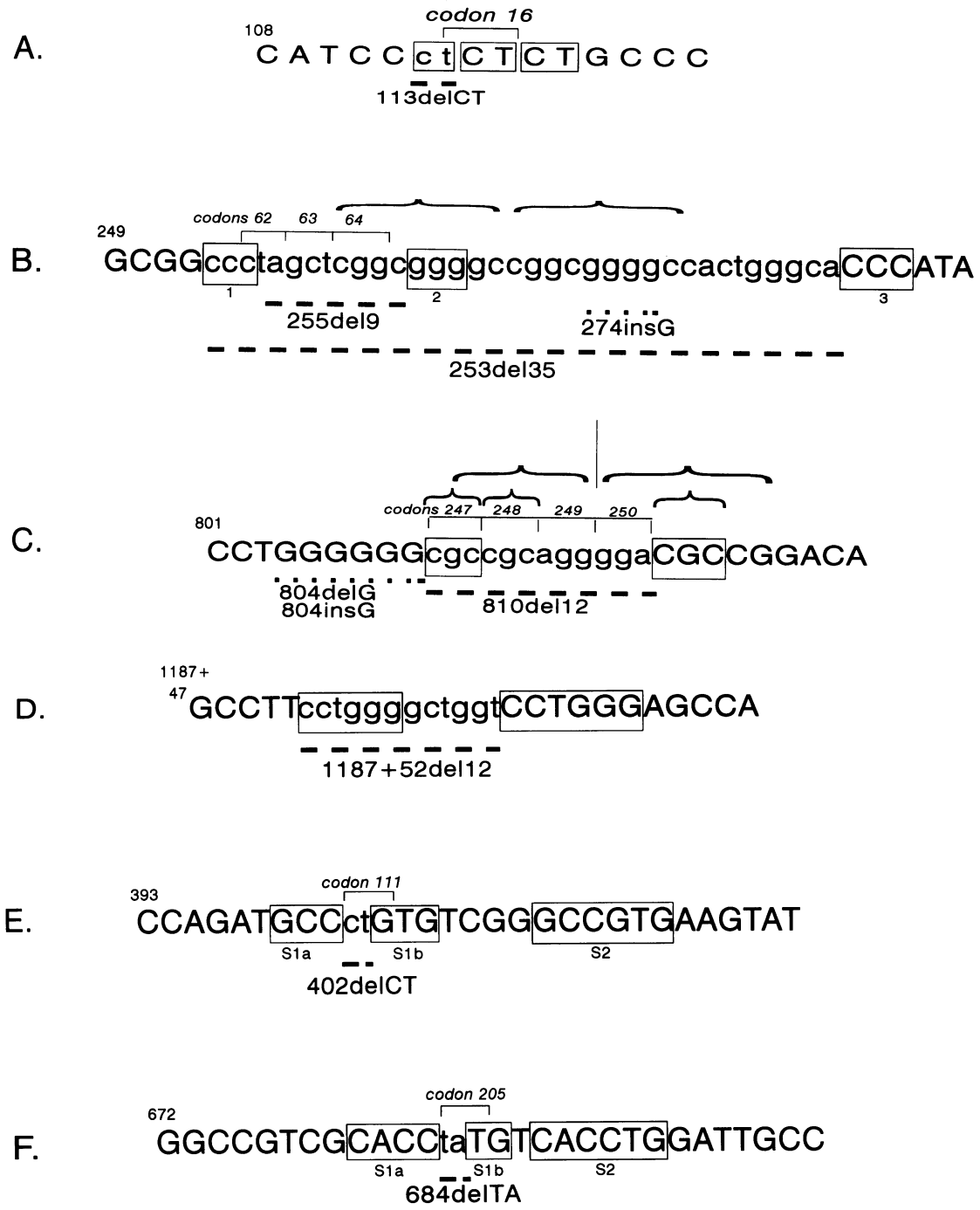

Figure 2 Proposed mechanisms of mutagenesis. Boxes enclose repeat units considered to be directly involved in the generation of a deletion. Longer dashes indicate a region where nucleotides were deleted, and shorter dashes indicate a region where a single nucleotide was inserted or deleted. The number over the first nucleotide in the sequence is the number according to the sequence of GenBank entry Z11687, which corresponds to the nucleotide position plus 71 bases in Birnbaumer et al. (1992, fig. 1). The codons are numbered according to the protocol of Birnbaumer et al. (1992). Horizontal braces above the sequence indicate other repeat units. *A*, 113delCT mutation that could result from slipped mispairing within repeated sequences. Deletion of a CT repeat could occur following a backward slip of the template strand during DNA replication (Sinden and Wells 1992, fig. 2). *B*, Slipped mispairing stabilized by a hairpin, which could generate the 255del9 mutation. Transient complementary base-pairing between box 1 and box 2 could form a hairpin stem; the 12 bp in the loop are excised; and the sequence is linearized and ligated (modified from Sinden and Wells 1992, fig. 3). Note that, by convention, the nucleotide position of the most 5′ nucleotide is used in the mutation name. For example, for purposes of nomenclature, nucleotides 255–263 (codons 62–64) are considered to be deleted. However, if the proposed mechanism is responsible for the deletion, nucleotides 256–264 would be deleted. The mechanism generating the 253del35 mutation is consistent with the slipped-mispairing model (Krawczak and Cooper 1991, fig. 5). The mechanism involves the misalignment, during DNA replication, of short direct repeats of 2–8 bp. If the direct repeat in box 3 forms base pairs with the complementary box 1 repeat, a single-stranded loop containing one of the repeats (box 1) and the intervening sequence between box 1 and box 3 is generated. Excision of the loop and rejoining of the sequence would generate the 35-bp deletion 253del35. The 274insG

Table 2

Xq28 NDI Haplotypes in Unrelated Families Carrying the Same AVPR2 Mutation

Family	AVPR2 Mutation	Xq28 Haplotype DXS52 (bp)	DXS15 (kb)	998A/G	G6PD[a]	F8C[b]
Q_1	R113W	1,690	5.8	A	2	1
Q_3	R113W	700	2.8	A	2	2
Holtzman et al. 1993*a*	R113W	1,690	5.8	A	1	1
Q_2	R137H	2,400	2.8	A	2	1
O_{15}	R137H	700	5.8	A	2	1
O_{16}	R137H	2,900	5.8	A	2	1
O_{23}, F_4 de novo	S167L	1,690	2.8	A	2	2
O_{12}	S167L	1,390	2.8	A	2	2
F_3 de novo[c]	S167L	1,300	5.8	G	2	2
O_{11}	R337X	1,300	5.8	A	2	2
F_1 de novo	R337X	700	5.8	A	1	1

[a] G6PD (1 = 400 bp; and 2 = 270 + 130 bp).
[b] F8C (1 = 320 bp; and 2 = 250 + 70 bp).
[c] Haplotype of maternal grandfather (origin of the S167L mutation) and mother; the affected male has a recombinant haplotype—1300 5.8 G 1 1.

R202C, and A285P), four nonsense mutations (Y124X, W284X, W293X, and R337X), three large deletions or gene rearrangements, one in-frame deletion (255del9), four other deletions (113delCT, 253del35, 402delCT, and 684delTA) and two insertions (274insG and 804insG) causing a frameshift and premature stop codon constitute a total of 26 different putative disease-causing mutations in 31 NDI families (table 1 and fig. 1). Of these, five mutations (R113W, Y128S, R137H, R181C, and R202C) have been identified elsewhere by us and others (R113W [Bichet et al. 1993; Holtzman et al. 1993*a*; Merendino et al. 1993*a*, 1993*b*], Y128S [Pan et al. 1992], R137H [Bichet et al. 1993], R181C [Pan et al. 1992], and R202C [van den Ouweland et al. 1992; Merendino et al. 1993*b*]). The R181C mutation was previously found in an allele also containing a 12-bp in-frame deletion in the third cytoplasmic loop (Pan et al. 1992). Since the NDI allele in family O_3 harbors only R181C, we suggest that it is sufficient to cause disease. Three large deletions all affecting the 3′ end of the gene are incompletely characterized. The extent of the deletions is ~1–2.5 kb; the breakpoints have not yet been sequenced.

In family O_8 three different mutations were identified in one NDI allele: a deletion mutation (402delCT) and two missense mutations, a Thr-to-Ser substitution at codon 7 and a Ala-to-Val substitution at codon 147. The deletion causes a frameshift 3′ to codon 111, which results in a premature stop codon at position 190; thus, this mutation is likely responsible for the disease, and the two missense mutations are provisionally considered to be sequence variants and are assigned the names "T/S7" and "A/V147." The two missense mutations occur in residues predicted to be in positions in the first extracellular and second cytoplasmic domain, positions at which amino acid substitutions may not seriously affect V_2 receptor function.

The mutation V88M has been ascribed, by Pan et al. (1992), to be a sequence variant. However, since it was the only mutation detected in the entire AVPR2 gene in the patient of family O_{13}, we tentatively consider it to be the disease-causing mutation.

AVPR2 sequence variants not causing NDI are also presented in table 1. The R/W64 missense mutation was present in the non-NDI chromosome of the father and his two daughters in family O_8. The 998A/G silent substitution first reported by Pan et al. (1992) was observed in 38% of 49 independent, normal AVPR2 alleles.

mutation could have occurred by a backward slip of the daughter strand during DNA replication. There are also a 9-bp tandem repeat and many other short repeats, which indicates that this region is likely to be a hot spot for deletion mutations. *C*, Mechanism generating the 810del12 mutation (Pan et al. 1992), which is also consistent with the slipped-mispairing model. The 804delG (Rosenthal et al. 1992) and the 804insC mutations could have been generated by slippage during DNA replication. There is an 8-bp symmetrical element and a 3-bp tandem and direct repeat (CGC), again indicating that this region could be a hot spot for deletion mutations. *D*, Deletion in the sequence 3′ to the stop codon (1187+52del12), which is another example of a deletion generated by the slipped-mispairing model. *E* and *F*, 402delCT and 684delTA mutations, both of which are examples of deletions generated by the modified slipped-mispairing model (Krawczak and Cooper 1991, fig. 6). Misalignment could occur between a contiguous sequence (box S2) on one strand and an interrupted homologous sequence on the other strand (box S1a and S1b in the complementary strand). Base pairing occurs when the nonhomologous nucleotides are looped out.

De Novo Mutations and Xq28 Haplotype Analysis

Eight de novo mutation events were detected by testing for the AVPR2 mutation in the parents, grandparents, and other relatives, when samples were available. Haplotype analysis was used to determine the origin of the de novo mutations. The origins of the mutant gametes were inferred to be maternal (274insG), grandmaternal (Y128S), and grandpaternal (R106C, S167L [twice], R202C, 684-delTA, and R337X).

Four mutations (R113W, R137H, S167L, and R337X) were each detected in more than one family (table 2). Three families (Q_1 and Q_3 [Bichet et al. 1993] and the family described by Holtzman et al. [1993*a*]) share the R113W mutation but have haplotypes with markers that differ on both sides of the AVPR2 gene. The three families who share the R137H mutation have different haplotypes on only one side of the AVPR2 gene. The S167L mutation was found on three different haplotypes: the de novo mutation in family F_4 arose on a common haplotype that was transmitted from the grandmother to the affected male in family O_{23}.

On the basis of haplotype analysis, a recombination event was detected or inferred in four families: between DXS15 and AVPR2 in two families (O_9 and O_{17}), between AVPR2 and G6PD in family F_3, and between DXS15 and G6PD in family Q_8. In the latter family, the affected male inherited a de novo mutation on a recombinant chromosome.

Discussion

We have identified 30 different AVPR2 mutations (fig. 1) that we consider to cause X-linked NDI in 37 families or extended kindreds (31 families in the present report, plus 6 families in previous reports [Rosenthal et al. 1992; Bichet et al. 1993]). The NDI patients in the many nuclear families of the Hopewell and Maritime kindreds share the W71X mutation identical by descent; therefore, we have counted this nonsense mutation only once. The NDI families constitute a worldwide collection, and the patients have a well-documented clinical phenotype. The majority of mutations described are point mutations and include 13 missense mutations, 6 nonsense mutations, 1 in-frame deletion, 5 deletions, and 2 insertions causing a frameshift and premature stop codon (Rosenthal et al. 1992; Bichet et al. 1993; present report). Three different large deletions or gene rearrangement were also partially characterized.

Evidence for Mutation Hot Spots and Mechanisms of Mutagenesis

The R113W, R137H, S167L, and R337X mutations were each found in at least two unrelated families. The S167L and R337X mutations were identified as de novo mutations or were found on different Xq28 haplotypes, which provides evidence that the appearance in each of the five families represents a recurrent mutation. The occurrence of the R113W and R137H mutation in each of three families on different Xq28 haplotypes (table 2) indicates two additional mutation hot spots. The mutations V88M, Y128S, R181C, and R202C have been reported in other, presumably unrelated families (Pan et al. 1992; van den Ouweland et al. 1992; Merendino et al. 1993*b*). Seven of these mutations (Y128S excluded) occurred at CpG dinucleotides that are mutation hot spots for genetic disease (Cooper and Krawczak 1990). Eight (27%) of 30 different NDI mutations were CG→TG or CG→CA changes. This occurred in 15 (41%) of 37 families. This high proportion is consistent with the relatively high proportion of CpG dinucleotides in the coding region of the AVPR2 gene, which has 58 (5%) CpG dinucleotides among 1,113 dinucleotides.

Small deletion and insertion mutations in the AVPR2 gene were all characterized by short direct repeats, complementary repeats, and/or symmetric sequences in the immediate vicinity of the mutation (fig. 2). This suggests that these deletions, like many others that have been described in other genes, resulted from slipped mispairing during DNA replication (Krawczak and Cooper 1991; Sinden and Wells 1992; Eng et al. 1993).

Clinical Aspects

It is notable that no significant variation in phenotypic expression (e.g., polyuric and polydipsic measurements, or urinary osmolality during dehydration) has been found in patients whom we have studied (Rosenthal et al. 1992; Bichet et al. 1993; present report), despite the different mutations encountered. In the course of our studies, we continued to investigate new families through two approaches: (1) mutation analysis of the AVPR2 gene by sequencing of the entire gene and (2) Xq28 haplotype analysis. The AVPR2 gene is relatively small (<2 kb) and contains three exons and two small introns. All the mutations found were confirmed by additional sequencing and/or restriction-enzyme–digestion analysis performed on other family members.

Xq28 haplotype analysis using markers that flank the AVPR2 gene has been useful to follow the segregation of the NDI chromosome prior to the identification of an AVPR2 mutation (Bichet et al. 1992, 1993), to identify the origin of a de novo mutation, and to obtain evidence for recurrent mutation. The opportunity for double recombination or two single-recombinant events in different meioses in the region between DXS52 and F8C that produce haplotypes with markers that differ on both sides of the AVPR2 gene harboring an NDI mutation is diminished because of selection against these deleterious alleles (Chakravarti 1992). We observed or inferred four single recombinants between (*a*) DXS52 and AVPR2 (twice), (*b*) DXS15 and G6PD, and (*c*) AVPR2 and G6PD. We continue to use haplotype analysis, preferably with the addi-

tion of informative markers in or very close to the AVPR2 gene, in parallel with DNA sequencing when perinatal testing is requested.

The family history of a patient with X-linked NDI usually discloses other affected male patients and/or males who died early in life with polyuric and polydipsic symptoms. However, in a family where a de novo mutation has occurred in a maternal or grandparental gamete, as described here, the proband may be the first affected male. As a consequence, the absence of a family history of X-linked NDI does not rule out the disease, and DNA of sporadic patients and their mothers should be analyzed for AVPR2 mutations. It is also necessary to consider the possibility of germ-line mosaicism, since this would increase the recurrence risk of NDI for brothers and maternal male cousins of an apparently sporadic case (Jeanpierre 1992). The results of mutation analysis of relevant members of the pedigree could provide information that might modify the calculated recurrence risk.

Prior knowledge of the AVPR2 mutation in NDI families has been of direct clinical value. For example, we obtained cord blood at birth from a male infant from family Q_2. The DNA was extracted and analyzed within 48 h; the newborn inherited the 2,400-bp St14 VNTR allele that segregated with the disease (Bichet et al. 1992). His AVPR2 gene was sequenced, and 48 h later it was confirmed that he had inherited the R137H mutation. The affected infant developed mild fever and hypernatremia (Na = 147 mEq/liter) 3 d after birth and was immediately treated with an abundant water intake, low-sodium milk, and subsequently with hydrochlorothiazide.

We encourage physicians who follow families with X-linked NDI to refer them for mutation analysis well before the birth of an at-risk male infant. The first manifestations of dehydration occur within the first days of life in affected male infants. The consequences of recurrent severe-dehydration episodes are physical and mental retardation or even death. However, early diagnosis and treatment of male infants affected with NDI can avert these deleterious consequences.

In summary, in the arginine-vasopressin V_2 receptor gene (AVPR2), we report 21 novel mutations that we consider to cause X-linked nephrogenic diabetes in 31 independent families, and we describe possible mechanisms of mutagenesis. Molecular carrier testing can be offered to female relatives who are at risk of having a male infant with NDI, and definitive diagnosis of male neonates can be accomplished in the majority of families, facilitating early treatment and prevention of significant morbidity and mortality. The in vitro expression of mutant AVPR2 sequences will lead to useful structure-function studies of the V_2 receptor.

Acknowledgments

We thank the patients and their families for their participation. We also thank Dr. E. J. Holtzman for providing us with DNA from the family described in the report by Holtzman et al. (1993*a*). This work was supported by grants MT-8126 and MA-9315 from the Medical Research Council of Canada, by the Canadian Kidney Foundation, and by the Canadian Genetic Diseases Network. W.R. and M.B. (grantee) were supported by grant DK-41244 from the National Institutes of Health. W.R. was recipient of a Heisenberg Fellowship from the Deutsche Forschungsgemeinschaft. D.G.B. is a Carrier Investigator of Le Fonds de la Recherche en Santé du Québec. G.N.H. is the recipient of a Scientist Award from the Medical Research Council of Canada.

References

Bichet DG (1992) Nephrogenic diabetes insipidus. In: Cameron JS, Davison AM, Grunfeld JP, Kerr DNS, Ritz E (eds) Oxford textbook of clinical nephrology. Oxford University Press, New York, pp 789–800

Bichet DG, Arthus M-F, Lonergan M, Hendy GN, Paradis AJ, Fujiwara TM, Morgan K, et al (1993) X-linked nephrogenic diabetes insipidus mutations in North America and the Hopewell hypothesis. J Clin Invest 92:1262–1268

Bichet DG, Hendy GN, Lonergan M, Arthus MF, Ligier S, Pausova Z, Kluge R, et al (1992) X-linked nephrogenic diabetes insipidus: from the ship Hopewell to RFLP studies. Am J Hum Genet 51:1089–1102

Bichet DG, Razi M, Lonergan M, Arthus MF, Papukna V, Kortas C, Barjon JN (1988) Hemodynamic and coagulation responses to 1-desamino[8-d-arginine]vasopressin in patients with congenital nephrogenic diabetes insipidus. N Engl J Med 318: 881–887

Birnbaumer M, Seibold A, Gilbert S, Ishido M, Barberis C, Antaramian A, Brabet P, et al (1992) Molecular cloning of the receptor for human antidiuretic hormone. Nature 357:333–335

Chakravarti A (1992) Fragile X founder effect? Nature Genet 1: 237–238

Cooper DN, Krawczak M (1990) The mutational spectrum of single base-pair substitutions causing human genetic disease: patterns and predictions. Hum Genet 85:55–74

Eng CM, Resnick-Silverman LA, Niehaus DJ, Astrin KH, Desnick RJ (1993) Nature and frequency of mutations in the α-galactosidase A gene that cause Fabry disease. Am J Hum Genet 53:1186–1197

Filosa A, Calabro V, Lania G, Vulliamy TJ, Brancati C, Tagarelli A, Luzzatto L, et al (1993) G6PD haplotypes spanning Xq28 from F8C to red/green color vision. Genomics 17:6–14

Holtzman EJ, Harris HW, Kolakowski LF, Guay-Woodford LM, Botelho B, Ausiello DA (1993*a*) A molecular defect in the vasopressin V2-receptor gene causing nephrogenic diabetes insipidus. N Engl J Med 328:1534–1537

Holtzman EJ, Kolakowski LF, O'Brien D, Crawford JD, Ausiello A (1993*b*) A null mutation in the vasopressin V2 receptor gene (AVPR2) associated with nephrogenic diabetes insipidus in the Hopewell kindred. Hum Mol Genet 2:1201–1204

Jeanpierre M (1992) Germinal mosaicism and risk calculation in X-linked diseases. Am J Hum Genet 50:960–967

Krawczak M, Cooper DN (1991) Gene deletions causing human genetic disease: mechanisms of mutagenesis and the role of the local DNA sequence environment. Hum Genet 86:425–441

McKusick VA (1990) Diabetes insipidus renal type I. In: Mendelian inheritance in man, 9th ed. Johns Hopkins University Press, Baltimore, pp 1585–1590

Merendino JJ Jr, Spiegel AM, Crawford JD, O'Carroll AM, Browstein MJ, Lolait SJ (1993*a*) A mutation in the vasopressin V2-receptor gene in a kindred with X-linked nephrogenic diabetes insipidus. N Engl J Med 328:1538–1541

Merendino JJ Jr, Thambi NC, Shenker A, Spiegel AM (1993*b*) Detection of V_2 vasopressin receptor gene mutations in X-linked nephrogenic diabetes insipidus by temperature gradient gel electrophoresis. Clin Res 41:131A

Pan Y, Metzenberg A, Das S, Jing B, Gitschier J (1992) Mutations in the V2 vasopressin receptor gene are associated with X-linked nephrogenic diabetes insipidus. Nature Genet 2:103–106

Reeves WB, Andreoli TE (1989) Nephrogenic diabetes insipidus. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed. McGraw-Hill, New York, pp 1985–2011

Rosenthal W, Antaramian A, Gilbert A, Birnbaumer M (1993) Nephrogenic diabetes insipidus: a V2 vasopressin receptor unable to stimulate adenylyl cyclase. J Biol Chem 268:13030–13033

Rosenthal W, Seibold A, Antaramian A, Lonergan M, Arthus MF, Hendy GN, Birnbaumer M, et al (1992) Molecular identification of the gene responsible for congenital nephrogenic diabetes insipidus. Nature 359:233–235

Schlessinger D, Mandel JL, Monaco AP, Nelson DL, Willard HF (1993) Report and abstracts of the Fourth International Workshop on Human X Chromosome Mapping 1993. Cytogenet Cell Genet 64:147–194

Seibold A, Brabet P, Rosenthal W, Birnbaumer M (1992) Structure and chromosomal localization of the human antidiuretic hormone receptor gene. Am J Hum Genet 51:1078–1083

Sinden RR, Wells RD (1992) DNA structure, mutations, and human genetic disease. Curr Opin Biotechnol 3:612–622

Tsukaguchi H, Matsubara H, Aritaki S, Kimura T, Abe S, Inada M (1993) Two novel mutations in the vasopressin V2 receptor gene in unrelated Japanese kindreds with nephrogenic diabetes insipidus. Biochem Biophys Res Commun 197:1000–1010

van den Ouweland AMW, Dreesen JCFM, Verdijk M, Knoers NVAM, Monnens LAH, Rocchi M, van Oost BA (1992) Mutations in the vasopressin type 2 receptor gene (AVPR2) associated with nephrogenic diabetes insipidus. Nature Genet 2: 99–102