A Novel G102E Mutation of *CYP27B1* in a Large Family with Vitamin D-Dependent Rickets Type 1

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Context: Mutations in the *CYP27B1* gene, which encodes vitamin D 1α-hydroxylase, are the genetic basis for vitamin D-dependent rickets type 1 (VDDR-I).

Objective: The aim of this study was to investigate the *CYP27B1* mutation in a large family with VDDR-I and characterize the genotype-phenotype correlation.

Patients and Methods: The index patient was a 23-yr-old female who had a progressive form of rickets and growth retardation since the age of 9 months. Laboratory data showed hypocalcemia, low urine calcium, hypophosphatemia, high serum alkaline phosphatase, elevated PTH, and low serum 1,25-dihydroxyvitamin D_3. Her parents were healthy first-degree cousins, and two of her 12 siblings were affected with similar but milder rickets. Three other siblings were asymptomatic but had biochemical evidence of the disease. The entire coding region of the *CYP27B1* gene was sequenced, and the mutation was characterized by functional studies.

Results: We found a novel biallelic c.305G>A sequence variation at codon 102, changing amino acid from glycine to glutamic acid (G102E) in the patient and five affected siblings, whereas a monoallelic c.305G>A variation was present in the mother and five nonaffected siblings. This variation was not present in 100 population controls. Expression of this mutant in CHO cells revealed an 80% reduction in the 1α-hydroxylase activity as compared to wild-type activity.

Conclusions: A novel mutation in the *CYP27B1* gene was found in patients with VDDR-I. This mutation resulted in a significant reduction in 1α-hydroxylase activity. The residual enzymatic activity may account for the mild phenotype presentation in some affected members. **(*J Clin Endocrinol Metab* 95: 4176–4183, 2010)**

Vitamin D consists of a group of biologically inactive, fat-soluble prohormones that exist in two major forms: ergocalciferol (vitamin D_2), derived from ergosterol after UV light exposure; and cholecalciferol (vitamin D_3), derived from animal tissues and from 7-dehydrocholesterol, which is formed in human skin by the action of UV rays in sunlight (1). Although ergosterol is produced by plants and fungi, there are few data to suggest that ergosterol is naturally converted to vitamin D_2 by UV light, and it is thus an artificial form of vitamin D. Both forms need two-step hydroxylation at carbons 25 and 1 for activation. The first step occurs in the liver, where vitamin D is hydroxylated to 25-hydroxyvitamin D [25(OH)D] by the hepatic 25-hydroxylase (1). At least three enzymes have 25-hydroxylase activity: mitochondrial *CYP27A1* (2), microsomal *CYP3A4* (3), and *CYP2R1* (4). The second step occurs mainly in the kidney, where 25(OH)D is hydroxylated by the mitochondrial vitamin D1α-hydroxylase to the biologically active hormone 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D], which binds to its nuclear receptor and achieves its biological activities (1, 5, 6). The renal synthesis of 1,25-$(OH)_2$D is tightly regulated by serum 1,25-

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Abbreviations: 1,25-$(OH)_2$D, 1,25-Dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; VDDR-I, vitamin D-dependent rickets, type 1; wt, wild-type.

TABLE 1. Clinical, laboratory, and genetic findings in a large family with 1α-hydroxylase deficiency

Subjects	Zygosity	Age (yr)/ sex	Clinical presentation	Ca (mmol/liter)	PO_4 (mmol/liter)	Mg (mmol/liter)	Alb (g/liter)	ALP (IU/liter)	25(OH)D (nmol/liter)	1,25-$(OH)_2$ D (pmol/liter)	PTH (ng/liter)	Urine Ca (mmol/24 h)
I-2 mother	Hetero	46/F	Asymptomatic	2.24	1	0.83	41	55 (30–125)	18	ND	64	ND
II-1	Homo	29/M	Bone pains, bowed legs, growth retardation	1.72	0.78	0.85	40	311 (40–135)	38	39.5[a]	366	0.9
II-2	Hetero	27/M	Asymptomatic	2.37	1.35	0.85	40	110 (40–150)	26	ND	35	0.48
II-3	Homo	26/F	Asymptomatic	2.19	0.77	0.8	43	228 (40–135)	56	78.5[a]	326	1.26
II-4 (index patient)	Homo	23/F	Bone pains, bowed legs, growth retardation	2.05	0.68	0.86	40	2177 (60–350)	38	18[a]	374	1.17
II-5	Hetero	22/F	Asymptomatic	2.48	1.27	0.98	39	55 (30–135)	42	124	65	0.82
II-6[b]	Homo	20/M	Asymptomatic	2.38	1.32	0.75	40	720 (60–350)	56	77	147	0.74
II-7[b]	Homo	20/M	Asymptomatic	2.34	1.24	0.77	39	732 (60–350)	66	46	196	ND
II-8	Hetero	19/M	Asymptomatic	2.5	1.61	0.93	43	225 (100–300)	63	ND	33	2.23
II-9	Homo	15/M	Bone pains, growth retardation	2.05	1.06	0.88	40	440 (100–300)	76	88.5[a]	137	0.79
II-10	Hetero	13/M	Asymptomatic	2.34	1.22	0.97	44	447 (60–350)	46	68	60	1.6
II-11	Wt	11/M	Asymptomatic	2.48	1.71	1.1	44	280 (100–240)	65	ND	43	1.37
II-12	Hetero	7/M	Asymptomatic	2.43	1.64	0.89	43	195 (100–300)	55	ND	24	ND
II-13	Wt	4/F	Asymptomatic	2.45	1.57	0.85	46	232 (100–240)	48	ND	43	ND
Normal ranges				2.1–2.6	1–1.5	0.7–1	35–45		27–90	38–133	10–65	2.5–6.3

Subject II-3 started taking calcium carbonate and calcitriol at age 21 yr. ALP is age-dependent, and the normal ranges are indicated in *parentheses.* ALP, Alkaline phosphatase; Alb, albumin; M, male; F, female; ND, not done.

[a] Subject was taking calcitriol and calcium at the time of testing.

[b] These two subjects are twins. They do not complain of any symptoms and are not taking any medications. They are of normal height and weight.

$(OH)_2D$, PTH, fibroblast growth factor 23, calcium, and phosphate, with renal 1α-hydroxylase being stimulated by PTH, hypophosphatemia, or hypocalcemia and inhibited by fibroblast growth factor 23 (1, 6, 7).

Disorders of vitamin D metabolism include vitamin D deficiency, resistance, and vitamin D biosynthesis, which includes mutations in *CYP27B1* and *CYP2R1* (1, 4, 6). These disorders result in the clinical and biochemical manifestations of rickets (1). There are two forms of vitamin D-dependent rickets, type 1 (VDDR-I) and type 2 (VDDR-II) (1). The latter is due to inactivating mutations in the vitamin D receptor (6). VDDR-I, also called pseudovitamin D deficiency rickets, is an autosomal recessive disorder caused by 1α-hydroxylase enzyme deficiency and characterized clinically by hypotonia, growth retardation, hypocalcemic seizures in early infancy, and radiographic features of rickets with typical laboratory findings such as hypocalcemia, elevated serum PTH, and low serum 1,25-$(OH)_2D$, despite normal or increased 25(OH)D (6, 8). Mutations in the *CYP27B1* gene are the molecular basis of VDDR-I (9). So far, at least 36 mutations in 54 patients from different ethnic groups have been reported (9–14). Certain mutations are more frequent in certain ethnic groups (14–16).

In the present report, we describe a large Saudi Arabian family with VDDR-I in whom we have identified a novel biallelic missense mutation in the *CYP27B1* gene in the affected family members. The effect of this mutation on the 1α-hydroxylase activity has been characterized. The genotype-phenotype correlation in this large family sheds

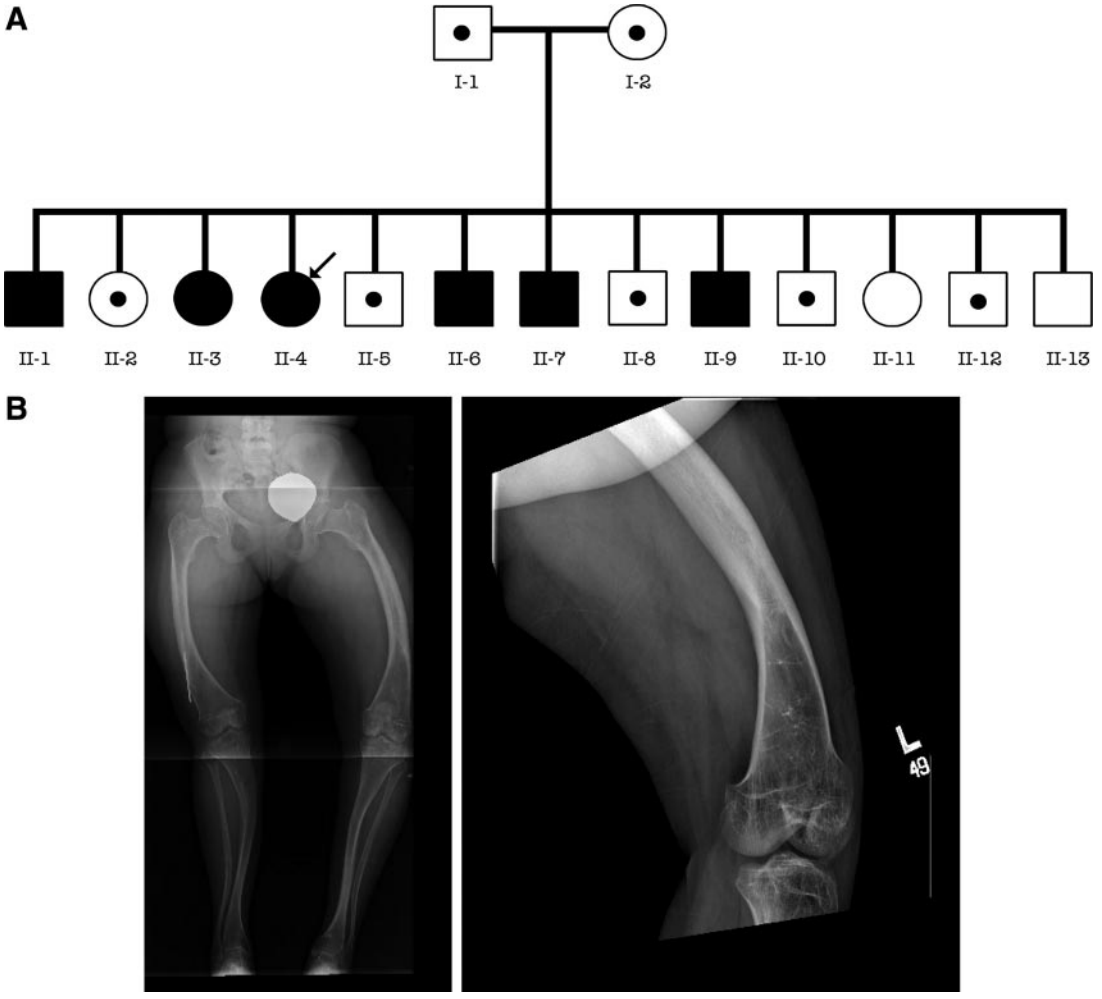

FIG. 1. Genetic study of a large family with pseudovitamin D deficiency rickets. A, Family pedigree. The index patient (II-4) and two (II-1 and II-9) of her 12 siblings had bone pain, growth retardation, and leg bowing at an early age. Other affected siblings (II-3, II-6, and II-7) had only an abnormal biochemical profile (Table 1). The remaining family members were healthy. Subject numbers are matched with those in Table 1. *Filled circles* (female) or *squares* (male) indicate affected subjects, *circles and squares with dots* indicate carriers, and *empty circles and squares* indicate normal subjects. B, Radiographs of the lower extremities with a detailed view of the index patient with VDDR-I. Note bilateral bowing and radiographic features of rickets, including splaying and threadlike shadows of calcification at the growth plate. C, Sequence analysis of human *CYP27B1* gene. Representative sequence chromatographs are shown. A biallelic c.305G>A (G102E) mutation was identified in all affected family members (II-1, II-3, II-4, and II-9 as shown in the pedigree and Table 1). A monoallelic c.305G>A mutation was found in two healthy brothers (II-2 and II-10). Subject II-11 is a normal boy. The *arrow* points to the G to A mutation in the exon 2. The parents are first-degree cousins. The father was not available for genetic evaluation but was predicted to be a carrier.

some light on the genetic and epigenetic factors influencing phenotype in this rare disorder.

Subjects and Methods

The index patient was a 23-yr-old woman who presented at age 16 yr with bone pains, significant growth retardation, and severe deformities of lower limbs. She had no history of seizures. At age 9 months, leg bowing and delayed growth were noted. Her condition gradually deteriorated over the years with worsening bowing of the legs, impaired walking with a waddling gait, and generalized bone pains. Her parents are healthy first-degree cousins. She had 12 siblings, two of whom were clinically affected but more mildly than she was (subjects II-1 and II-9; Table 1), and three others were found to have only biochemical abnormalities consistent with VDDR-I (subjects II-3, II-6, and II-7; Table 1). The remaining seven siblings had no symptoms. The family pedigree is shown in Fig. 1A. Physical examination revealed severe growth retardation (height, 122 cm; weight, 35 kg), moderate proximal myopathy, severe bilateral outward leg bowing, and kyphoscoliosis. She was pubertal with Tanner stage IV breast, genitalia, and pubic hair development. Her laboratory data and family members are shown in Table 1. Radiographic findings of her rickets are shown in Fig. 1B. The patient and her affected siblings were treated with calcitriol 0.5–1 mg twice daily and calcium carbonate 600–1200 mg twice daily. All showed gradual but remarkable improvement with complete normalization of calcium, phosphate, alkaline phosphatase, and PTH over 2–3 yr of treatment. The current study is approved by the Institutional Review Board of the King Faisal Specialist Hospital and Research Centre, and informed consent was obtained from patients.

Genomic DNA isolation

Genomic DNA from peripheral blood leukocytes was isolated using the Gentra Blood Kit (QIAGEN Corp., Valencia, CA).

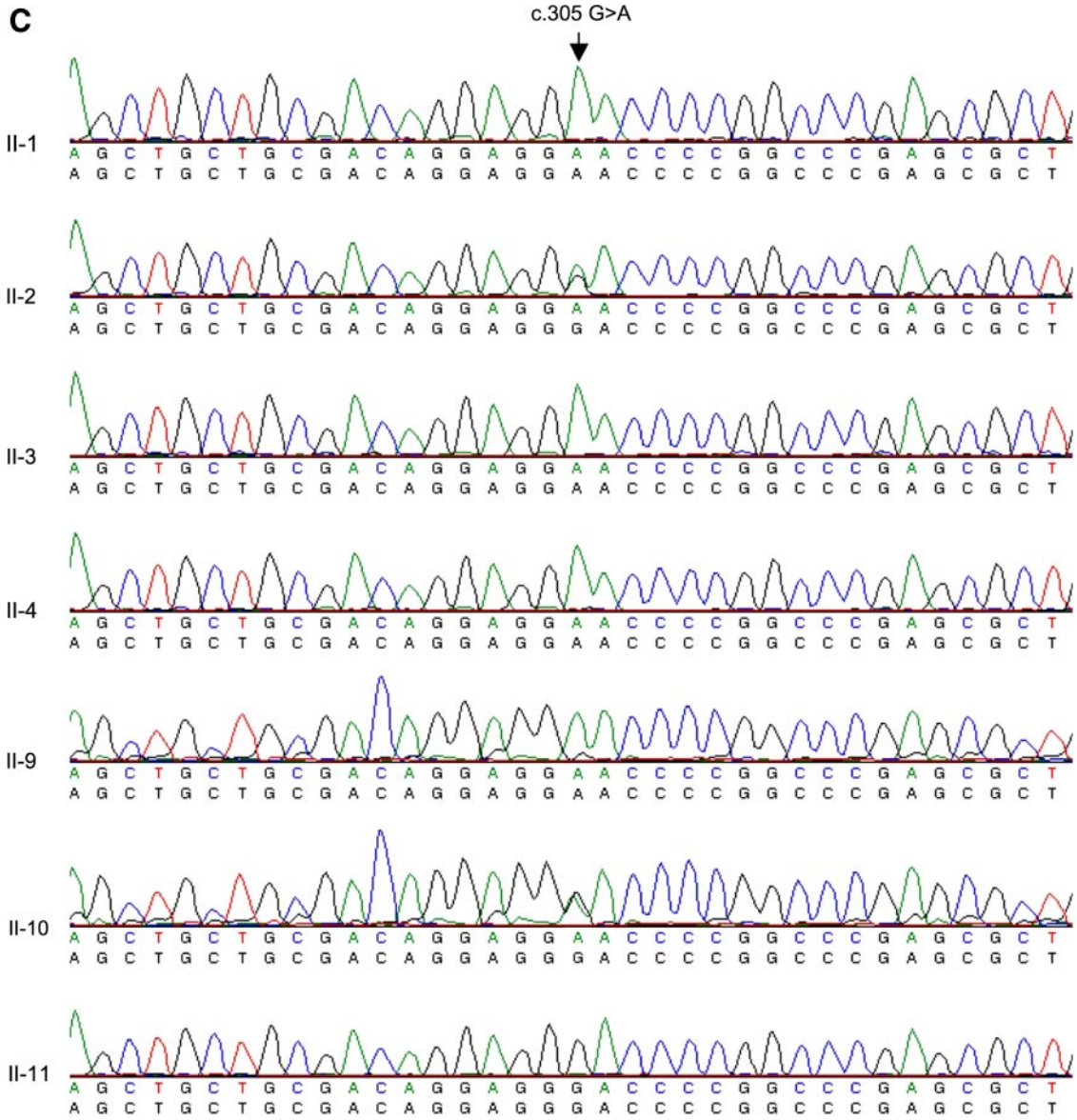

FIG. 1. (*Continued*).

DNA amplification and sequencing

All nine exons and intron-exon boundaries of *CYP27B1* were amplified by PCR from 100 ng of genomic DNA. The PCR primers are listed in Supplemental Table 1 (published on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org); PCR conditions were 95 C for 5 min, followed by 35 cycles of amplification (95 C for 30 sec, 54 C for 30 sec, and 72 C for 30 sec). The resulting PCR products were directly sequenced using a BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA).

Site-directed mutagenesis, cDNA cloning, and expression

The wild-type (wt) *CYP27B1* cDNA was ordered from GeneCopoeia Inc. (Rockville, MD) and was used as a template for the creation of G102E mutant by PCR-based overlap extension mutagenesis (17). Two overlapping PCR fragments were amplified using two primer sets, with set 1 amplifying the 310-bp N-terminal part of the gene (forward, 5′-CCAGACCATGACCCAGACCCTCAA-3′; reverse, 5′-GGGTTCCTCCTGTCGCAGCAGCT-3′; the translation start codon and mutated base are *underlined*) and set 2 amplifying the 1250-bp C-terminal part (forward, 5′-AGCTGCTGCGACAGGAGGAACCC-3′; reverse, 5′-CTACTATCTGTCCAAAAACTGT-3′, the translation stop codon and mutated base are *underlined*). The PCR conditions were the same as described above, and the resulting two fragments were gel purified. Two microliters of each fragment were mixed together and reamplified by PCR using the following primers: forward, 5′-CCAGACCATGACCCAGACCCTCAA-3′; and reverse, 5′-CTACTATCTGTCCAAAAACT GT-3′. The mutation in the 1.5-kb full length cDNA fragment was verified by sequencing. Both wt and mutant cDNAs were subsequently cloned into pcDNA3.1 expression vector (Invitrogen Co., Carlsbad, CA), and stably transfected into CHO cells. The stable clones were pooled for gene expression as described previously (18). T321R mutant was created in a similar way, which was reported to have no enzymatic activity (12).

Analysis of 1α-hydroxylase activity

CHO cells stably transfected with wt, G102E, and T321R were seeded in six-well plates overnight in growth medium and incubated in serum-free medium with 1 μM saturating concentration of 25(OH)D_3 (Sigma, St. Louis, MO) for 1 h and 4 h, respectively. The Km for wt *CYP27B1* is 0.28 μM (19). The concentration of 1,25-$(OH)_2D_3$ in the medium was measured by RIA according to the manufacturer's procedure (Immunodiagnostic Systems Inc., Fountain Hills, AZ).

Western blot analysis

Sixty micrograms of protein were loaded into a 12% SDS-polyacrylamide gel. Proteins were transferred to a polyvinylidene difluoride membrane and probed with *CYP27B1* antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Results

Sequence analysis of the *CYP27B1* gene

The diagnosis of 1α-hydroxylase deficiency was made in the index patient and affected family members based on their clinical and biochemical features (Table 1). To identify the underlying molecular defect, we sequenced the entire coding region and intron-exon boundaries of the *CYP27B1* gene in the patient and her five affected siblings. A biallelic c.305G>A sequence variation at codon 102 (G102E in exon 2) was found in all of them. Subsequent sequence analysis of exon 2 from the remaining eight non-affected family members revealed a monoallelic c.305G>A in five siblings and the mother (Fig. 1 and Table 1) and a wt sequence in two siblings. This sequence variation has not been reported in the literature or among 34 known NCBI *CYP27B1* single nucleotide polymorphisms (www.genecards.org). To rule out this variation as a novel polymorphism, we screened 100 population controls and did not find a c.305G>A variation. Thus, it is likely that the c.305G>A is a novel mutation.

1	refNP_000776.1	human (homo sapiens)	...AAPALVEELLRQE G PRPERCSFSPWTE...
2	ref|XP_509175.2|	chimpanzees [Pan troglodytes]	...AAPALVEELLRQE G PRPERCSFSPWTE...
3	ref|XP_001116450.1|	rhesus monkeys (Macaca mulatta)	...AAPALVEELLRQE G PRPERCSFSPWTE...
4	ref|XP_588481.1|	cattle (Bos taurus)	...AAPTLVEQLLRQE G PRPERCSFSPWTE...
5	ref|NP_999160.1|	pig [Sus scrofa]	...AAPTLVEQLLRQE G PLPERCSFSPWTE...
6	ref|XP_538254.2|	dog (Canis lupus familiaris)	...AAPALVEQLLRQE G PRPERCSFSPWAE...
7	ref|XP_001490036.1|	horse [Equus caballus]	...AAPTLIEQLLRQE G PRPERCSFSSWAE...
8	ref|XP_001380742.1|	opossum (Monodelphis domestica)	...AAPALIEQLLRQE G PHPERCSFSPWVE...
9	dbj|BAA22434.1|	mouse (Mus musculus)	...ADPTLVEQLLRQE S HCPERCSFSSWAE...
10	ref|NP_446215.1|	rat (Rattus norvegicus)	...ADPALVEQLLRQE S HCPERCSFSSWSE...
11	ref|XP_422077.2|	red jungle fowl [Gallus gallus]	...ADRDMVAQVLRSE G RAPQRANMESWQE...
12	gb|AAH77308.1|	frog [Xenopus laevis]	...GDPEALQQLLRQE G KYPMRNKEDIWKA...
13	gb|AAH94536.1|	Xenopus tropicalis	...ASPELLETLLRQE G KYPMRTDMFMWKE...

FIG. 2. Partial protein alignment of *CYP27B1* gene from human, monkey, cattle, dog, rat, mouse, and frog around position 102. The glycine residue at the 102 is conserved across different species, except that the serine residue is present in mouse and rat instead. Glycine (G) is a nonpolar hydrophobic amino acid, whereas glutamic acid (E) is a polar acidic hydrophilic amino acid. The G102E mutation may change the secondary or tertiary structure of 1α-hydroxylase and disrupt its function.

Protein secondary structure change

Glycine (G) is a nonpolar hydrophobic amino acid, whereas glutamic acid (E) is a polar acidic hydrophilic amino acid. The G102E mutation may change the secondary or tertiary structure of 1α-hydroxylase and disrupt its function. To predict the possible impact of the G102E substitution on the structure and function of 1α-hydroxylase, we first performed multiple protein sequence alignments around codon 102 across different species to see whether it is conserved. As shown in Fig. 2, the glycine at codon 102 is relatively conserved, except that serine was present in mouse and rat instead. Next, we used two protein structure prediction programs [PSIPRED, Protein Structure Prediction Server (http://bioinf.cs.ucl.ac.uk/psipred) and PolyPhen (http://genetics.bwh.harvard.edu/pph/)] to predict the change of protein secondary structure. PSIPRED predicted two significant changes in the protein structure caused by G102E, which is located in a loop followed by B-helix and is in agreement with previous studies (20) (Supplemental Fig. 1), whereas PolyPhen did not.

Characterization of functional consequence of G102E mutant on 1α-hydroxylase activity in CHO cells

Although the G102E mutation was predicted to be benign by PolyPhen, the clinical and laboratory data suggest that 1α- hydroxylase activity was reduced in our patients. To confirm whether the G102E mutant has any functional effect on 1α-hydroxylase activity, we generated a G102E mutant. Both mutant and wt plasmids were transfected into CHO cells for stable expression. As shown in Fig. 3A, 1α- hydroxylase protein was expressed in CHO^{G102E} and CHO^{wt} cells. Subsequently, 1α- hydroxylase activity was measured by its ability to convert 25(OH)D_3 into 1,25-$(OH)_2D_3$ in CHO^{G102E} and CHO^{wt} cells. As shown in Fig. 3B, during 4-h incubation with 25(OH)D_3, 1,25-$(OH)_2D_3$ produced by CHO cells transfected with vector alone was similar to that in culture medium (22.7 $\pm$ 3.9 *vs.* 17.7 $\pm$ 1.5 fmol/10^5 cells), indicating that CHO cells have no 1α-hydroxylase activity (the background value was thus subtracted for calculating enzymatic activity). During 1-h incubation with 25(OH)D_3, CHO^{wt} produced 255.3 $\pm$ 7.4 fmol/10^5 cells of 1,25-$(OH)_2D_3$, compared with 52 $\pm$ 2.1 fmol/10^5 cells in CHO^{G102E}. Therefore, about 20% enzymatic activity was retained in the G102E mutant. With further incubation with 25(OH)D_3 for up to 4 h, CHO^{wt} produced 1080 $\pm$ 127 fmol/10^5 cells of 1,25-$(OH)_2D_3$ (4-fold increase) *vs.* 193.3 $\pm$ 12 fmol/10^5 cells by CHO^{G102E} (3.6-fold increase), which accounted for 17.9% of wt activity. T321R did not show any enzymatic activity (Fig. 3B). These data confirm that G102E had lost at least 80% of its activity and was the cause of VDDR-I in the affected family members.

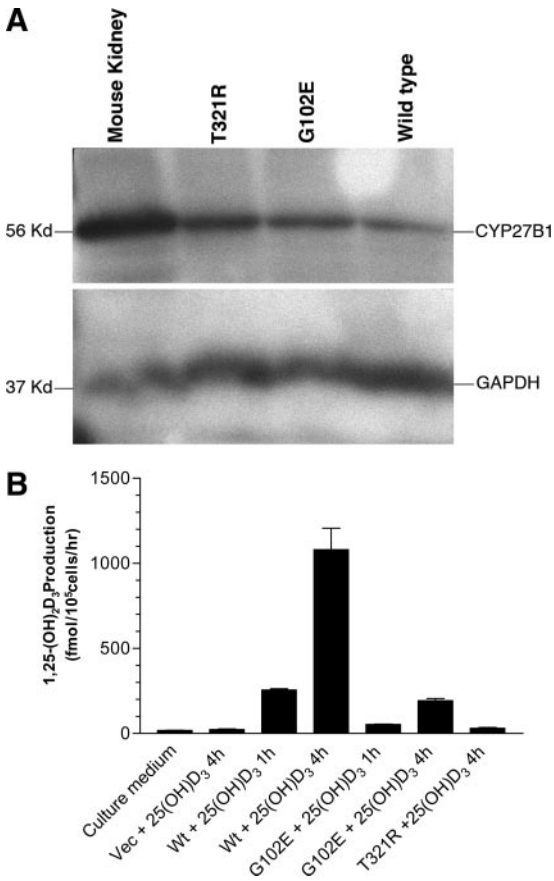

FIG. 3. 1α-Hydroxylase gene expression and activity in CHO cells expressing G102E, T321R, or wt *CYP27B1* cDNA. A, Western blot analysis of 1α-hydroxylase protein expression in mouse kidney, CHO^{G102E}, CHO^{T321R}, and CHO^{wt}. The *CYP27B1* protein (56 Kd) was detected by a rabbit antihuman *CYP27B1* antibody. The membrane was reprobed with a glyceraldehyde-3-phosphate dehydrogenase (37 Kd) antibody to monitor protein loading. B, The G102E, T321R, and wt cDNA were stably transfected into CHO cells as well as vector control. The cells were incubated with 1 μM 25(OH)D_3 in 2 ml serum-free medium for 1 and 4 h, respectively. The concentration of 1,25-$(OH)_2D_3$ in the medium was measured by RIA. Data are expressed as means $\pm$ SEM of three separate experiments. 1α-Hydroxylase activity was not observed in CHO cells transfected with vector alone or T321R. The G102E mutant retained 20% of wt activity during 1 h incubation with 25(OH)D_3 as demonstrated by 80% reduction of 1,25-$(OH)_2D_3$ in the medium compared with the wt.

Discussion

In this report, we have described a large family with VDDR-I. The index patient had severe deformities of the legs, growth retardation, hypocalcemia, hypophosphatemia, marked elevation of alkaline phosphatase, and secondary hyperparathyroidism with normal 25(OH)D but low 1,25-$(OH)_2D$. These features are consistent with the diagnosis of VDDR-I. All the affected members carry a novel biallelic G102E, whereas unaffected members are

either normal or heterozygous for the mutation. These data strongly suggest that G102E is the underlying molecular defect, which was confirmed by subsequent *in vitro* functional studies.

The human *CYP27B1* gene that encodes 1α-hydroxylase was mapped to chromosome 12q14 in 1990 by linkage analysis (21) and was subsequently cloned in 1997 (9, 22, 23), whereas the rat *CYP27B1* counterpart was cloned a bit earlier in the same year (22, 24). It is approximately 5 Kb and is composed of nine exons and eight introns (25). The G102E was very close to previously reported R107H (10). The other two reported mutations located in exon 2 are P112L and G125E (10, 14). All of these mutations reside in the substrate recognition site 1 (19, 26) except G102E. Homology modeling of human *CYP27B1* based on the crystal structure of rabbit *CYP2C5* suggests that R107H and G125E would disrupt protein folding (27). Because R107 has been described as a heme-binding residue (28) and is very close to G102, G102E may disrupt heme incorporation or heme functioning. It could also disrupt the placement of the B-helix or the β-3 sheet, both of which are critical to positioning the heme and the heme-binding loop containing the catalytic cysteine (20).

Among 22 missense mutations found in human *CYP27B1* (14), 18 occurred in amino acid residues common to both human and mouse, suggesting that these residues are important for enzymatic activity. However, there are four mutations located in residues that are not conserved: P112 in human *vs.* S112 in mouse, G102 *vs.* S102, T409 *vs.* S408 (the mouse is one amino acid short compared with human), and R429 *vs.* N428. The changes from hydrophobic amino acids proline (P) or glycine (G) to neutral amino acid serine (S), neutral amino acid threonine (T) to serine, and basic hydrophilic amino acid arginine (R) to neutral amino acid asparagine (N) may not significantly affect protein structure and function. For example, S408T has the lowest effect on 25(OH)D 1α hydroxylation activity among S408T, S408A, S408V, and S408I mutants compared with wt; and S408I has the most detrimental effect (19). Indeed, the human T409I (equivalent to mouse S408I) has no 1α-hydroxylase activity (16). However, one should also consider the overall structural and functional context unique to each mutation to predict function. The G102E, although not conserved between human and mouse (S102), lost 80% of enzymatic activity as a result of a significant structural change. Given the presence of S102 in mouse and rat and G102 in human, which probably fooled PolyPhen into predicting a benign structural change, we expect that G102S would cause a much milder effect than G102E.

With the exception of three cases of mild VDDR-I caused by three different mutations (E189G, E189K, and L343F) in which some partial *in vitro* 1α-hydroxylase activity was present (13), there was a complete absence of *in vitro* enzymatic activity in all the cases described (12, 16). Among the reported mutants that have residual 1α-hydroxylase activity (13), E189G retains 22% of wt activity compared with E189K (11%) and L343F (2.3%). The G102E described in the present study retains 20% activity. Although these mutations correlate well with mild clinical and laboratory presentation, no enzymatic activity has been reported in patients with mild clinical manifestations (12). It has been suggested that the phenotype of VDDR-I could be modified by endogenous or exogenous factors in addition to the *CYP27B1* mutation (12). The large family presented here offered a unique opportunity for studying genotype-phenotype correlation. Except for the index patient who presented with relatively severe clinical manifestation, the other five affected siblings had mild to moderate manifestations (Table 1). It is interesting to mention that two affected siblings (subjects II-6 and II-7) are twins who had only mild abnormal biochemical profiles. Given that the index patient had no history of seizures or bone fracture, her disease severity can be considered relatively mild compared with other severe cases reported in the literature (12, 14). The phenotype variation of these patients may be due to epigenetic factors (12). Clearly, our data and those from Wang *et al.* (13) indicate relatively good genotype-phenotype correlation and show that the residual enzymatic activity may contribute to the mild phenotype.

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References

1. **Holick MF** 2007 Vitamin D deficiency. N Engl J Med 357:266–281
2. **Miller WL, Portale AA** 2001 Genetics of vitamin D biosynthesis and its disorders. Best Pract Res Clin Endocrinol Metab 15:95–109
3. **Gupta RP, He YA, Patrick KS, Halpert JR, Bell NH** 2005 CYP3A4 is a vitamin D-24- and 25-hydroxylase: analysis of structure function by site-directed mutagenesis. J Clin Endocrinol Metab 90:1210–1219
4. **Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW** 2004 Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci USA 101:7711–7715

5. **Miller WL** 2005 Minireview: regulation of steroidogenesis by electron transfer. Endocrinology 146:2544–2550
6. **Miller WL, Portale AA** 2003 Vitamin D biosynthesis and vitamin D 1 α-hydroxylase deficiency. Endocr Dev 6:156–174
7. **Liu S, Quarles LD** 2007 How fibroblast growth factor 23 works. J Am Soc Nephrol 18:1637–1647
8. **Fraser D, Kooh SW, Kind HP, Holick MF, Tanaka Y, DeLuca HF** 1973 Pathogenesis of hereditary vitamin-D-dependent rickets. An inborn error of vitamin D metabolism involving defective conversion of 25-hydroxyvitamin D to 1 α,25-dihydroxyvitamin D. N Engl J Med 289:817–822
9. **Fu GK, Lin D, Zhang MY, Bikle DD, Shackleton CH, Miller WL, Portale AA** 1997 Cloning of human 25-hydroxyvitamin D-1 α-hydroxylase and mutations causing vitamin D-dependent rickets type 1. Mol Endocrinol 11:1961–1970
10. **Kitanaka S, Takeyama K, Murayama A, Sato T, Okumura K, Nogami M, Hasegawa Y, Niimi H, Yanagisawa J, Tanaka T, Kato S** 1998 Inactivating mutations in the 25-hydroxyvitamin D3 1α-hydroxylase gene in patients with pseudovitamin D-deficiency rickets. N Engl J Med 338:653–661
11. **Miller WL, Portale AA** 1999 Genetic disorders of vitamin D biosynthesis. Endocrinol Metab Clin North Am 28:825–840, x
12. **Kitanaka S, Murayama A, Sakaki T, Inouye K, Seino Y, Fukumoto S, Shima M, Yukizane S, Takayanagi M, Niimi H, Takeyama K, Kato S** 1999 No enzyme activity of 25-hydroxyvitamin D3 1α-hydroxylase gene product in pseudovitamin D deficiency rickets, including that with mild clinical manifestation. J Clin Endocrinol Metab 84:4111–4117
13. **Wang X, Zhang MY, Miller WL, Portale AA** 2002 Novel gene mutations in patients with 1α-hydroxylase deficiency that confer partial enzyme activity *in vitro*. J Clin Endocrinol Metab 87:2424–2430
14. **Kim CJ, Kaplan LE, Perwad F, Huang N, Sharma A, Choi Y, Miller WL, Portale AA** 2007 Vitamin D 1α-hydroxylase gene mutations in patients with 1α-hydroxylase deficiency. J Clin Endocrinol Metab 92:3177–3182
15. **Miller WL, Portale AA** 2000 Vitamin D 1 α-hydroxylase. Trends Endocrinol Metab 11:315–319
16. **Wang JT, Lin CJ, Burridge SM, Fu GK, Labuda M, Portale AA, Miller WL** 1998 Genetics of vitamin D 1α-hydroxylase deficiency in 17 families. Am J Hum Genet 63:1694–1702
17. **Aiyar A, Xiang Y, Leis J** 1996 Site-directed mutagenesis using overlap extension PCR. Methods Mol Biol 57:177–191
18. **Shi Y, Zou M, Collison K, Baitei EY, Al-Makhalafi Z, Farid NR, Al-Mohanna FA** 2006 Ribonucleic acid interference targeting S100A4 (Mts1) suppresses tumor growth and metastasis of anaplastic thyroid carcinoma in a mouse model. J Clin Endocrinol Metab 91:2373–2379
19. **Yamamoto K, Uchida E, Urushino N, Sakaki T, Kagawa N, Sawada N, Kamakura M, Kato S, Inouye K, Yamada S** 2005 Identification of the amino acid residue of CYP27B1 responsible for binding of 25-hydroxyvitamin D3 whose mutation causes vitamin D-dependent rickets type 1. J Biol Chem 280:30511–30516
20. **Prosser DE, Guo Y, Jia Z, Jones G** 2006 Structural motif-based homology modeling of CYP27A1 and site-directed mutational analyses affecting vitamin D hydroxylation. Biophys J 90:3389–3409
21. **Labuda M, Morgan K, Glorieux FH** 1990 Mapping autosomal recessive vitamin D dependency type I to chromosome 12q14 by linkage analysis. Am J Hum Genet 47:28–36
22. **St-Arnaud R, Messerlian S, Moir JM, Omdahl JL, Glorieux FH** 1997 The 25-hydroxyvitamin D 1-α-hydroxylase gene maps to the pseudovitamin D-deficiency rickets (PDDR) disease locus. J Bone Miner Res 12:1552–1559
23. **Monkawa T, Yoshida T, Wakino S, Shinki T, Anazawa H, Deluca HF, Suda T, Hayashi M, Saruta T** 1997 Molecular cloning of cDNA and genomic DNA for human 25-hydroxyvitamin D3 1 α-hydroxylase. Biochem Biophys Res Commun 239:527–533
24. **Shinki T, Shimada H, Wakino S, Anazawa H, Hayashi M, Saruta T, DeLuca HF, Suda T** 1997 Cloning and expression of rat 25-hydroxyvitamin D3–1α-hydroxylase cDNA. Proc Natl Acad Sci USA 94:12920–12925
25. **Fu GK, Portale AA, Miller WL** 1997 Complete structure of the human gene for the vitamin D 1α-hydroxylase, P450c1α. DNA Cell Biol 16:1499–1507
26. **Gotoh O** 1992 Substrate recognition sites in cytochrome P450 family 2 (CYP2) proteins inferred from comparative analyses of amino acid and coding nucleotide sequences. J Biol Chem 267:83–90
27. **Yamamoto K, Masuno H, Sawada N, Sakaki T, Inouye K, Ishiguro M, Yamada S** 2004 Homology modeling of human 25-hydroxyvitamin D3 1α-hydroxylase (CYP27B1) based on the crystal structure of rabbit CYP2C5. J Steroid Biochem Mol Biol 89–90:167–171
28. **Prosser DE, Jones G** 2004 Enzymes involved in the activation and inactivation of vitamin D. Trends Biochem Sci 29:664–673