Novel *CYP27B1* Gene Mutations in Patients with Vitamin D-Dependent Rickets Type 1A

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The *CYP27B1* encodes 25-hydroxyvitamin D-1α-hydroxylase. Mutations of the gene cause vitamin D-dependent rickets type 1A (VDDR-IA), which is a rare autosomal recessive disorder. To investigate *CYP27B1* mutations, we studied 8 patients from 7 unrelated families.

Table 1. Clinical, laboratory, and genetic findings of 7 families with VDDR-I

Family	Subjects	Clinical features	Time point	Age	Height (SDS)	Ca (mg/dL)	P (mg/dL)	ALP (IU/L)	25OHD (ng/ml)	1,25(OH)$_2$ D (pg/mL)	PTH (ng/L)	Mutation	Consanguinity
I	I-1 Father	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1022-1037del16	Yes Families I and II are not related.
	I-2 Mother	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1022-1037del16	
	I-3 Son	Inability to walk	At diagnosis	16 months	-2.5	8.5	3.4	1802	40.44	3.2	703.8	Biallelic c.1022-1037del16	
			Most recent	25 months	-2.87	9.5	3.4	1131	ND	ND	195.3		
II	II-1 Father	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1022-1037del16	No, but parents from the same village
	II-2 Mother	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1022-1037del16	
	II-3 Son	Failure to thrive and inability to walk	At diagnosis	17 months	-2.85	8.9	1.94	1523	189	9.1	560	Biallelic c.1022-1037del16	
			Most recent	25 months	-2.87	9.1	3.2	638	52.8	ND	136.6		
III	III-1 Father	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1215+2T>A	No
	III-2 Mother	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1215+2T>A	
	III-3 Son	Inability to walk	At diagnosis	21 months	-4.13	**6.5**	2.9	1622	125	25	319	Biallelic c.1215+2T>A	
			Most recent	8 years	-2.45	9.4	4.6	226	23.1	ND	28		
IV	IV-1 Father	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.195+2T>G	Yes
	IV-2 Mother	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.195+2T>G	
	IV-3 Son	Failure to thrive and inability to walk	At diagnosis	12 months	-2.54	8.9	1.8	2190	44	4.5	938	Biallelic c.195+2T>G	
			Most recent	16 months	-3.29	8.7	2.2	1879	ND	ND	998		
	IV-4 Daughter	Failure to thrive and inability to walk	At diagnosis	26 months	-5.22	**7.1**	2.7	1850	35	<2.1	466	Biallelic c.195+2T>G	
			Most recent	8 years	-5.28	9.2	5.2	343	40.7	ND	138		
V	V-1 Father	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.195+2T>G	No
	V-2 Mother	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.195+2T>G	
	V-3 Daughter	Failure to thrive and fractures	At diagnosis	21 months	-3.96	8.6	2.5	1825	238	14	728	Biallelic c.195+2T>G	
			Most recent	31 months	-3.72	10.2	4.1	432	41.3	ND	37.7		
VI	VI-1 Father	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1215+2T>A	Yes. Families III and VI are not related.
	VI-2 Mother	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.1215+2T>A	
	VI-3 Daughter	Failure to thrive, fractures, and blue sclera	At diagnosis	13 months	-4.33	**4.2**	3.5	684	40	ND	284	Biallelic c.1215+2T>A	
			Most recent	12 years	-4.5	9.0	5	232	31.7	ND	217		
VII	VII-1 Father	normal				ND	ND	ND	ND	ND	ND	Monoallelic c.934_935delAC	Yes
	VII-2 Mother	normal				9.4	4	86	12.2	50	50	Monoallelic c.934_935delAC	
	VII-3 Daughter	Hypocalcemic convulsion	At diagnosis	13 months	ND	**6.5**	3.9	1100	54	13	555	Biallelic c.934_935delAC	
			Most recent	25 months	-1.3	9.6	4	350	ND	ND	40		
Normal range						8.8-10.6	3.7-6.8	82-380	20-100	17-53	15-65		

ND: not done; SDS: standard deviation score or Z-score
SI unit conversions: to convert the values for 25OHD to nmol/L, multiply by 2.5; to convert the values for 1,25(OH)2D to pmol/L, multiply by 2.4; to convert the value for calcium to mmol/L, divide by 4; to convert the values for phosphate to mmol/L, divide by 3.1.

All coding exons and intron-exon boundary of *CYP27B1* gene were amplified by PCR from peripheral leukocyte DNA and subsequently sequenced. Biallelic mutations in the *CYP27B1* gene were found in all the patients and monoallelic mutations were present in their normal parents.

Four novel mutations were identified: A 16-bp deletion in exon 6 (c.1022-1037del16, p.T341Rfs*346) (Figure 1), a splice donor site mutation (c.1215+2T>A) in intron 7 (Figure 2), a 2-bp deletion in exon 5 (c.934_935delAC, p.T312Rfs*331) (Figure 3) and c.1215 T>C (p.R379R) in the last nucleotide of exon 7 (Figure 2).

Clinically, all the patients required continued calcitriol treatment and the clinical presentations were consistent with the complete loss of vitamin D1α-hydroxylase activity.

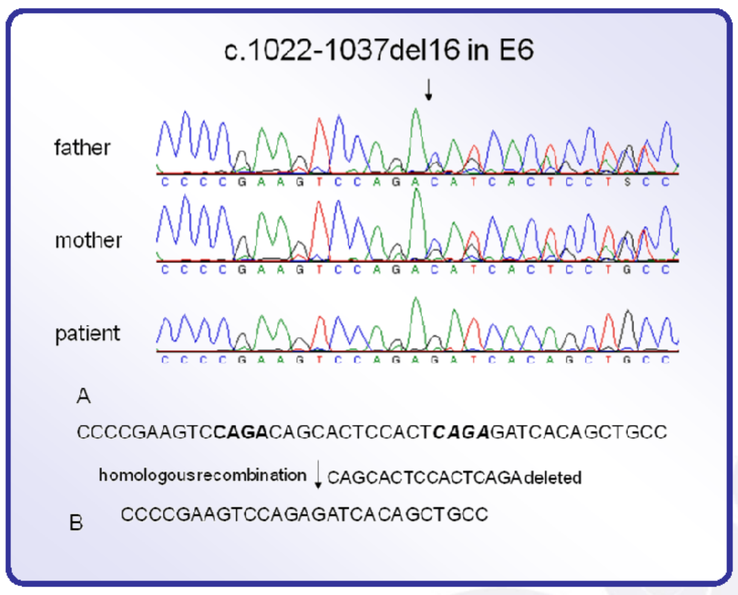

Fig. 1. A novel deletion of 16-bp nucleotides in the human *CYP27B1* gene. (A) Sequence analysis shows a biallelic deletion of 16-bp nucleotides in exon 6 in a patient from family 1. Both of his parents carry a monoallelic deletion. (B) A schematic representation of the deletion. The deleted nucleotide sequence is underlined and the 4-bp nucleotide repeats flanking the deleted sequence are highlighted in bold.

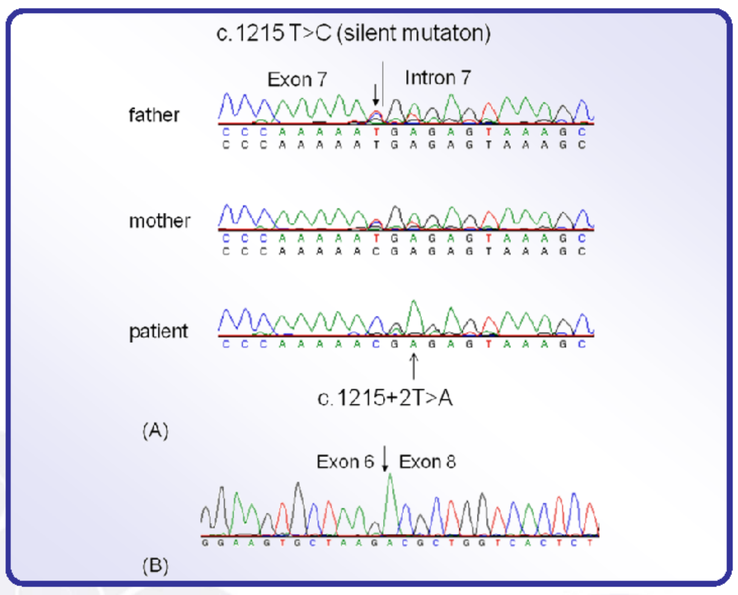

Fig. 2. Novel splice site mutations in the human *CYP27B1* gene. (A) Sequence analysis of genomic DNA from peripheral lymphocytes. A biallelic mutation at the splice donor site of intron 7 (c.1215+2T>A) were found in a patient from family 3. A biallelic silent mutation (c.1215 T>C) at the end of exon 7 was also identified. His parents carry a monoallelic mutation at the both locations. The mutations are indicated by arrows. (B) Sequence analysis of cDNA from patient's peripheral lymphocytes. The mutation at the c.1215+2T>A leads to skipping of exon 7, resulting in exons 6 and 8 joined together.

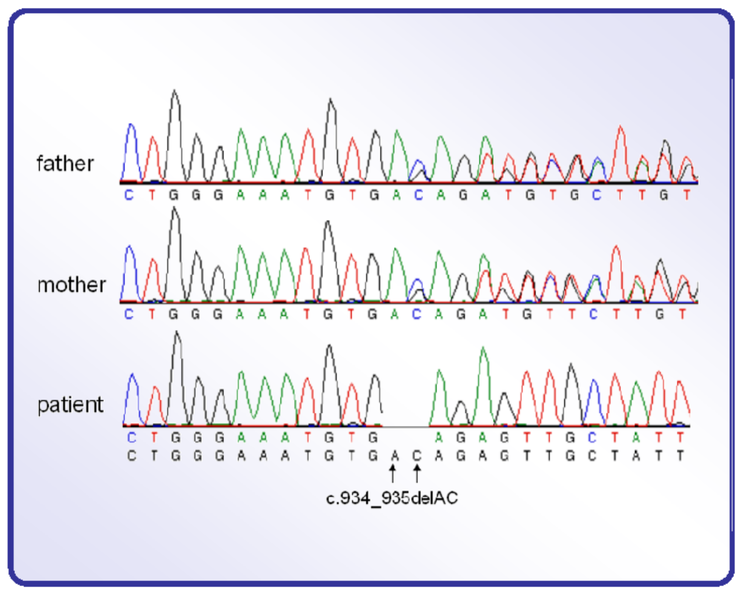

Fig. 3. A novel deletion of 2-bp nucleotides in the human *CYP27B1* gene. A biallelic deletion of 2-bp nucleotides (c.934_935delAC) in exon 5 was found in a patient from family 7. A monoallelic deletion was found in both of his parents. The deletion results in a frameshit and creates a premature TGA stop codon at amino acid 331 (p.T312RfsX331).

In conclusion, four novel mutations have been identified. Three of them caused frameshift and truncated proteins. The silent c.1215 T>C has no effect on pre-mRNA splicing and may be considered as a novel SNP. The current study further expands the *CYP27B1* mutation spectrum.

ESPE 2015 239--P2
Bone, Growth Plate and Mineral Metabolism
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DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: ESPE 2015 BARCELONA European Society for Paediatric Endocrinology
PosterSessionOnline.com