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-1A), which is a rare autosomal recessive disorder. To investigate CYP27B1 mutations, we studied 8 patients from 7 unrelated families.

<table>
<thead>
<tr>
<th>Family</th>
<th>Subject</th>
<th>Clinical features</th>
<th>Time-point</th>
<th>Age</th>
<th>Height (SDS)</th>
<th>Ca (mg/dL)</th>
<th>Phosphorus (mg/dL)</th>
<th>ALP (KAL)</th>
<th>25(OH)D (ng/mL)</th>
<th>1,25(OH)2D (ng/mL)</th>
<th>PTH (ng/mL)</th>
<th>Mutation</th>
<th>Consanguinity</th>
</tr>
</thead>
</table>
| I      | Father  | normal           | At diagnosis | 16 months | -2.5 | 0.5 | 802 | 40.44 | 3.2 | 703.8 | Yes | Monogenic c.1022-1037del16
|        |         |                  | Most recent | 25 months | -2.87 | 9.5 | 1131 | No | 193.3 | Monogenic c.1022-1037del16
| I      | Mother  | normal           |            |       |             |            |                   |          |                |                   |            | Bi-allelic c.1022-1037del16
|        |         |                  |            |       |             |            |                   |          |                |                   |            | Bi-allelic c.1022-1037del16
| II     | Father  | normal           | At diagnosis | 17 months | -2.85 | 8.9 | 1023 | 189 | 9.1 | 560 | No, but parents from the same village |
|        | Mother  | normal           |            |       |             |            |                   |          |                |                   |            | Monogenic c.1215+2T-A
|        |         |                  |            |       |             |            |                   |          |                |                   |            | Monogenic c.1215+2T-A
| II     | Father  | normal           | At diagnosis | 21 months | -4.13 | 6.5 | 29 | 122 | 25 | 319 | No |
|        | Mother  | normal           |            |       |             |            |                   |          |                |                   |            | Monogenic c.1215+2T-A
|        |         |                  |            |       |             |            |                   |          |                |                   |            | Monogenic c.1215+2T-A
| IV     | Father  | normal           | At diagnosis | 12 months | -2.54 | 8.9 | 2190 | 44 | 4.5 | 938 | Yes | Monogenic c.195+2G
|        | Mother  | normal           |            |       |             |            |                   |          |                |                   |            | Monogenic c.195+2G
|        |         |                  |            |       |             |            |                   |          |                |                   |            | Monogenic c.195+2G
| IV     | Daughter | failure to thrive and inability to walk | At diagnosis | 16 months | -3.29 | 0.7 | 1879 | No | 998 | No |
|        |         |                  |            |       |             |            |                   |          |                |                   |            | Monogenic c.195+2G
| V      | Father  | normal           | At diagnosis | 8 years | -2.68 | 9.2 | 343 | 40.7 | 138 | -1.9 | No |
|        | Mother  | normal           |            |       |             |            |                   |          |                |                   |            | Monogenic c.195+2G
|        | Daughter | failure to thrive and fractures | At diagnosis | 11 months | -3.96 | 8.6 | 2199 | 234 | 14 | 728 | No |
|        |         |                  |            |       |             |            |                   |          |                |                   |            | Monogenic c.195+2G
| V      | Father  | normal           | At diagnosis | 13 months | -3.72 | 10.3 | 413 | 413 | 37.7 | Yes |
|        | Mother  | normal           |            |       |             |            |                   |          |                |                   |            | Monogenic c.1215+2T-A
|        | Daughter | failure to thrive and fractures, and blue sclera | At diagnosis | 12 years | -4.5 | 9.0 | 232 | 317 | 217 | No |
|        |         |                  |            |       |             |            |                   |          |                |                   |            | Monogenic c.594_595delAC
| V      | Father  | normal           | At diagnosis | 13 months | -3.13 | 6.5 | 390 | 54 | 13 | 555 | Yes |
|        | Mother  | normal           |            |       |             |            |                   |          |                |                   |            | Monogenic c.594_595delAC
|        | Daughter | Hypocalcemic convulsion | At diagnosis | 13 months | -3.13 | 6.5 | 390 | 54 | 13 | 555 | Yes |

Normal range: 8.0-10.6 3.7-4.8 82-326 22-100 17-63 15-45

No: not done; SDS: standard deviation score or Z-score
SI unit conversions: to convert the values for 25(OH)D to nmol/L, multiply by 2.0; to convert the values for 1,25(OH)2D to pmol/L, multiply by 4.2; to convert the value for calcium to mmol/L, divide by 4.0; to convert the values for phosphate to mmol/L, divide by 3.1.

All coding exons and intron-exon boundary of CYP27B1 exon 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.594_595delAC, p.T312Rfs*331) (Figure 3) and c.1215 T>C (p.R373X) 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 D1-alpha-hydroxylase activity.

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.