TWO FRENCH FAMILIES WITH VITAMIN D DEPENDENCY RICKETS TYPE 1B HARBOR HOMOZYGOUS RECESSIVE EXPRESSION OF CYP2R1 MUTATIONS L99P and G42_L46delinsR.

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Introduction: Mutations of CYP2R1 (11p15.2) encoding the main vitamin D 25-hydroxylase have been associated with a rare recessive autosomal form of rickets, also called vitamin D dependency rickets type 1B (VDDR-1B) (Cheng et al. 2004). We describe rickets & loss-of-function CYP2R1 mutations in 6/10 individuals tested from two unrelated families: five patients in family 1 (F1) with homozygous L99P mutations and one boy in family 2 (F2) with a novel homozygous mutation G42_L46delinsR. Calcidiol (25-(OH)D3) therapy resulted in complete normalization of biochemical and bone defects.

Methods: Clinical and routine laboratory explorations: (Ca and P, PTH, alkaline phosphatase (ALP) and vitamin D metabolites) Data were collected at the time of the diagnosis, retrospectively and prospectively, using records from hospitals. Molecular analysis: CYP2R1 exons 1-5 and their intron-exon junctions were sequenced using standard procedures on a Beckman Coulter DNA Sequencer. Functional study: The mutations, as well as another variant M248I found in the French population, were recreated and tested using an in vitro mammalian expression system described previously (JBiolChem 286:28729).

Results: radiological findings, were typical of rickets. All affected children presented with similar biochemical findings: hypocalcemia, hypo/normo phosphatemia, high PTH and ALP levels. While serum 1,25-(OH)2D levels were within the normal range (F1: II13) or even high (F2: II2), 25(OH)D3 was undetectable.

(F1) II-3 and II-4: genu valgum during infancy treated with 25-OH-D3; adulthood: normocalcemia, slightly elevated PTH, normal 1,25-(OH)2D and undetectable 25-OH-D3 levels
II-1: limb amyotrophy and genu valgum
II-2: genu valgum
II-3: asymptomatic

(F2) Clinical (A), radiological (B) and biochemical findings in II2
II-2: short stature, genu varum, hypotonia; initially treated with high doses of alfcalcidol (1α-OH D3) and calcium supplementation: serum calcium normalized, but PTH and ALP levels remained elevated.

Molecular analysis: Sequence analysis of CYP2R1 in the probands revealed the previously described c.296T>C (L99P) mutation in the exon 2 in F1, and a novel c.124_138delGCGG (p.G42_L46delinsR) in the exon 1 in F2. Mutations were in a homozygous state in both probands, and in a heterozygous state in their parents. The brother and the sister of the probands in F1 also carried the L99P mutation in a homozygous state.

Functional study: L99P and G42_L46delinsR showed <5% of wild type CYP2R1 enzyme activity and are presumed to be loss-of-function mutations, while the M248I variant had 75% activity and is thus likely a polymorphism.

Method used to analyse enzymatic activity of CYP2R1 (A), (B) results of LC/MS-MS expressed relative to the wild-type activity. (D) expression of each variant

CYP2R1 deficiency should be investigated in patients presenting rickets with low/undetectable 25-OH-D serum concentration even so 1,25(OH)2D3 concentration is normal. The precise identification of the genetic defect allowed an appropriate therapy resulting in complete normalization of bone defect and biochemical parameters.