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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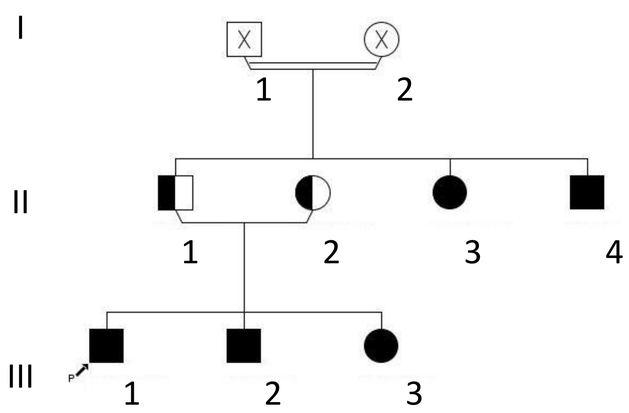
Introduction: Mutations of *CYP2R1* (11p15.2) encoding the main vitamin D 25-hydroxylase have been associated with a rare recessive autosomal form of rickets, also called vitamin D dependency rickets type 1B (VDDR-1B) (Cheng et al. 2004).
We describe rickets & loss-of-function *CYP2R1* mutations in 6/10 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.
Calcifediol (25-OH D_3) 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-$(OH)_2$D levels were within the normal range (F1 : III3) or even high (F2 : II2), 25(OH)D was undetectable:

(F1)

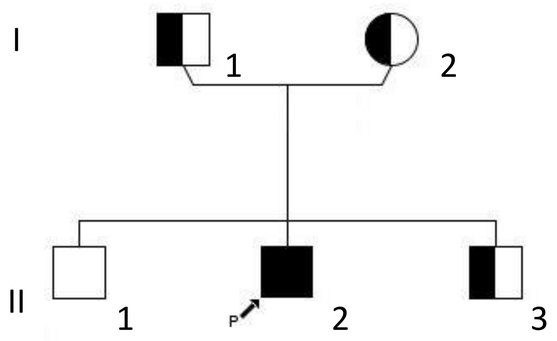


II-3 and II-4: genu valgum during infancy treated with 25-OH D_3; adulthood: normocalcemia, slightly elevated PTH, normal 1,25-$(OH)_2$D and undetectable 25-OH D levels
III-1: limb amyotrophy and genu valgum
III-2: genu valgum
III-3: asymptomatic

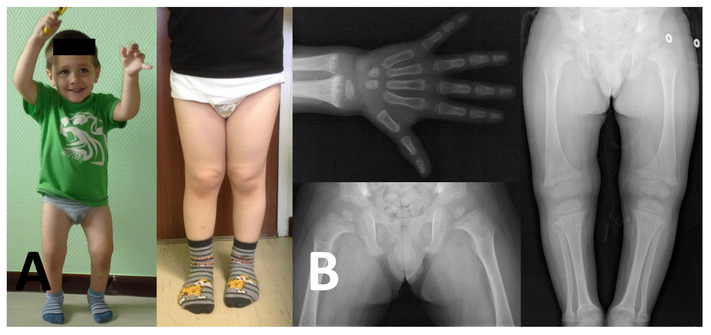
	Calcium 2,20-2,65 mmol/L	Phosphate 1,29-2,26 mmol/L	ALP 36-315 UI/L	PTH_{1-84} 6,5-36,5 pg/mL	25-OH D >50 ng/mL	1,25-$(OH)_2$D 65-135 pmol/L
III-1 9 years	**1.98**	**1.07**	**1921**	**338**	**<4**	**36**
III-2 7 years	**1.38**	**1.29**	**1117**	**345**	**<4**	**64**
III-3 4 years	**2.45**	**1.42**	**266**	**130**	**<4**	**117**

(F2)

Clinical (A), radiological (B) and biochemical findings in II2



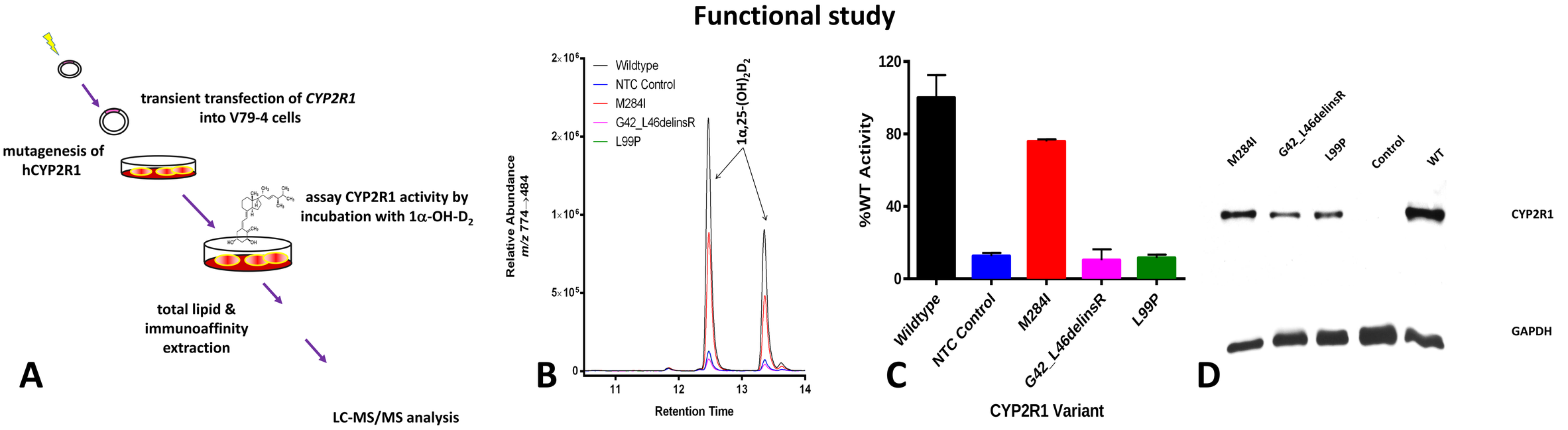
II-2: short stature, genu varum, hypotonia; initially treated with high doses of alfacalcidol (1α-OH D_3) and calcium supplementation: serum calcium normalized, but PTH and ALP levels remained elevated.



Calcium (2.20-2.65 mmol/L)	1.6
Phosphate (1.29-2.26 mmol/L)	1.4
ALP (36-315 UI/L)	762
PTH_{1-84} (6.5-36.5 pg/mL)	216
25-OH-D (>50 nmol/L)	<10
1,25-$(OH)_2$D (65-135 pmol/L)	194

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_138delinsCGG (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 M284I variant had 75% activity and is thus likely a polymorphism.

Functional study



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)2D concentration is normal. The precise identification of the genetic defect allowed an appropriate therapy resulting in complete normalization of bone defect and biochemical parameters.**

Conflicts of interest: none

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