Loss of function *CYP24A1* mutations in patients with hypercalcemia and low PTH level: an autosomal dominant or recessive trait

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Abstract

Homozygous or compound heterozygous mutations of the gene *CYP24A1* coding vitamin D 24-hydroxylase have recently been reported to cause Idiopathic Infantile Hypercalcemia (IIH) due to increased intestinal absorption of calcium [1]. However, an autosomal dominant transmission with partial penetrance of the trait was also suggested [2]. So far, only case-reports have been published. Frequency of *CYP24A1* mutations in hypercalcemic patients remains unknown.

Here we describe a cohort of patients presenting with hypercalcemia and low PTH rate, to better define the phenotype of patients who should benefit of *CYP24A1* genetic screening and to evaluate the frequency of the disease.

In addition, we also show that simultaneous measurement of vitamin D metabolites by liquid chromatography-tandem mass spectrometry (LC-MS/MS) is a valuable screening tool for these patients.

Objectives

1) **To evaluate the frequency of *CYP24A1* mutation in hypercalcemic patients with low PTH rate**
2) **To study the impact of *CYP24A1* heterozygous mutation on calcium metabolism**
3) **To highlight the usefulness of LC/MSMS for vitamin D metabolites measurement**

References

1-Schlingmann *et al.* 2011 *N Engl J Med* 365:410-21

2-Tebben P *et al. 2011*, *J Clin Endocrinol Metab* 97:E423-E7

3-Kaufmann M *et al.* , 2014. *J Clin Endocrinol Metab* 99:2567-2574.

Methods

Patients

We studied 72 index cases presenting with hypercalcemia (>2.6 mmol/L) and low PTH levels (<20pg/mL) and 22 heterozygous relatives.

Biochemical parameters

Data on clinical symptoms, renal ultrasound examination and biological explorations were collected at the time of the diagnosis, or retrospectively using records from hospitals or primary care physicians.

Simultaneously assay of vitamin D metabolites

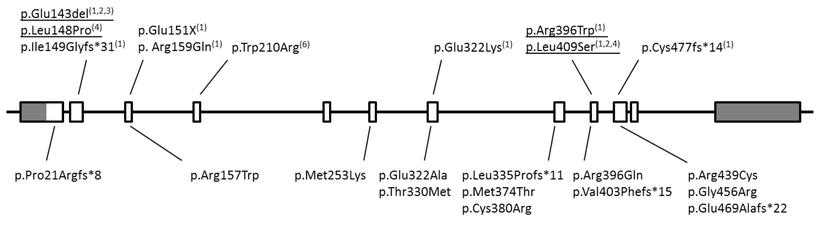
LC-MS/MS analysis was performed at the time of molecular diagnosis as previously described [3] including 25-OH D_3 and 24,25-$(OH)_2D_3$ using 100µl of serum; results are expressed as a ratio of 25-OH D_3:24,25-$(OH)_2D_3$. Values under 25 indicated no defect in 24-hydroxylase activity and were considered as normal.

Molecular analysis

11 coding exons of *CYP24A1* and their intron-exon junctions were sequenced as previously described (Castanet et al 2013). New variations of sequence interpreted according to pathogenicity prediction programs (PolyPhen-2, Align-GVGD, MutationTaster, SIFT).

Results 1

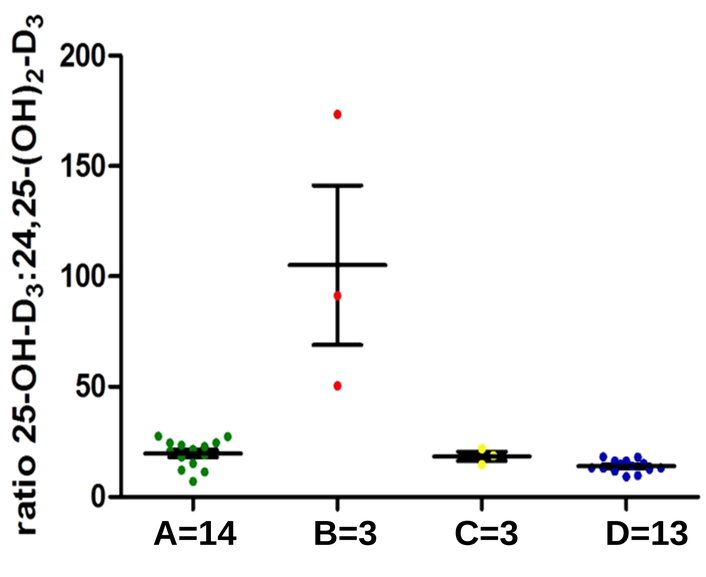
We identified 25 patients (35%) harboring mutations in coding sequence of *CYP24A1* : 20 patients (28%) with bi-allelic mutations (10 homozygous, and 10 compound heterozygous) and 5 children with heterozygous mutation (7%). All were neonates, under 2 weeks (range 1 to 13 days). In these patients, hypercalcemia was found during routine exams performed for another pathology: prematurity, growth retardation, infection or apnea. None presented with renal pathology



Positions of observed mutations in the human *CYP24A1* gene.

Results 2

Patients with bi-allelic *CYP24A1* mutations (group B) exhibit a dramatic increase in 25-OH D_3:24,25-$(OH)_2D_3$ ratio (105 [48.8-173.4]) providing evidence "*in vivo*" for the loss of CYP24A1 enzyme activity. By contrast, 25-OH D_3:24,25-$(OH)_2D_3$ ratio remains within the normal range (R= 19.7[7-27.5]) in probands without *CYP24A1* mutation (group A).



A : no mutation
B : bi-allelic mutation
C : heterozygous index
D : heterozygous relatives

In patients heterozygous for *CYP24A1* mutations, probands (group C) as well as relatives (group D), simultaneous assay of both 25-OH D_3 and 24,25-$(OH)_2D_3$ provides evidence for the presence of normal CYP24A1 activity with a 25-OH D_3:24,25-$(OH)_2D_3$ ratio within the normal range (R=13.7 [9.3-18]).

Conclusions

We identified mutation in *CYP24A1* as a major cause of hypercalcemia associated to low PTH level.

We confirm the accuracy and effectiveness of a novel blood test estimating the ratio between relevant vitamin D metabolites 25-OH D_3 and 24,25-$(OH)_2D_3$. This test constitutes a useful screening tool.

We suggest that in patients with *CYP24A1* haplo-insufficiency, vitamin D supplementation associated with a low renal function could trigger hypercalcemia and hypercalciuria.

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