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The impact of functional characterization of variants in calcium sensing receptor gene (*CASR*) on the clinical diagnosis

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Introduction

The human calcium-sensing receptor (CaSR) is a G-protein-coupled receptor that signals *via* intracellular calcium mobilization and MAPK pathway to regulate extracellular calcium homeostasis. Loss- or gain-of-function heterozygous *CASR* variants lead to familial hypocalcaemic hypercalcaemia type 1 (FHH1) or autosomal-dominant hypocalcaemia (ADH), respectively.

Material and methods

We analysed the *CASR* gene in eleven unrelated families presenting abnormalities of the calcium and phosphate metabolism. The missense variants of unknown significance were induced by site-directed mutagenesis into a wild-type (WT) CaSR-expressing plasmidic vector. HEK293 cells were transfected with either the WT, the variant or cotransfected with both CaSR-containing vectors. We conducted two luciferase reporter gene assays using NFAT and SRE response elements to measure the function of CaSR on the two major CaSR-associated pathways.

Results

Eleven heterozygous *CASR* genetic variants were identified in eight different FHH1 families, one ADH family and one patient presenting nephrolithiasis and hypercalcaemia. In families I and J we identified two different variants *in cis* (complex allele) and *in trans* (compound heterozygote), respectively (Table 1). The variant didn't cosegregate with the phenotype for the H family.

The functional assays on MAPK pathway allowed to classify the eleven variants as :

- gain of function (1/11)
- loss of function (6/11 + 1 complex allele with 2 variants)
- without impact (2/11) (Figure 1)

The Ca²⁺_i pathway were concordant with these results. Co-transfections : absence of negative dominant effect.

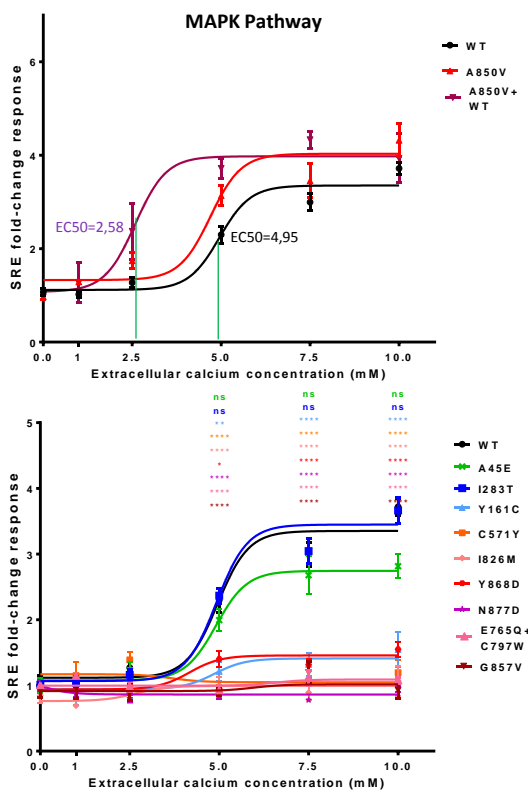
Family (cases)	Phenotype	Variant	
		Nucleotide HTZ	Protein
A (2)	ADH	c.2549C>T	p.(Ala850Val) (A850V)
B (1)	Nephrolithiasis	c.134C>A	p.(Ala45Glu) (A45E)
C (1)	FHH1	c.482A>G	p.(Tyr161Cys) (Y161C)
D (1)	FHH1	c.1712G>A	p.(Cys571Tyr) (C571Y)
E (3)	FHH1	c.2478C>G	p.(Ile826M) (I826M)
F (1)	FHH1	c.2602T>G	p.(Tyr868Asp) (Y868D)
G (1)	FHH1	c.2629A>G	p.(Asn877Asp) (N877D)
H (1)	FHH1	c.848T>C	p.(Ile283Thr) (I283T)
I (1)	FHH1	c.848T>C	p.(Ile283Thr) (I283T)
		c.1972C>T (<i>trans</i>)	p.(Arg658*) (R658X)
J (2)	FHH1	c.2293G>C	p.(Glu765Gln) (E765Q)+
		c.2391C>G (<i>cis</i>)	p.(Cys797Trp) (C797W)
K (1)	FHH1	c.2570G>T	p.(Gly857Val) (G857V)

Table 1. Clinical and genetic data for 11 families.

ADH = autosomal dominant hypocalcaemia;
FHH = familial hypocalcaemic hypercalcaemia type 1;
HTZ = heterozygous.

Discussion and conclusion

- ✔ Study of 11 families with clinical and genetic heterogeneity.
- ✔ The protocol is effective to detect the gain of function, loss of function and polymorphic variants.
- ✔ The co-transfection variant/WT is important to reproduce the HTZ state *in vitro*, especially for gain of function variants or complex.
- ✔ The functional studies are important for the clinical interpretation, especially in complex situations.
- ✔ Clinical impact : choice of medical management, genetic counselling in the affected families.



**** p<0,0001; *** p<0,001; ** p<0,01; * p<0,05, ns = non significant, two-way ANOVA multiple comparisons with Tukey post-hoc analysis.

Figure 1. Effects of the 11 variants on MAPK signaling. Concentration-response curve of Ca²⁺_i-induced SRE-luciferase reporter response. Statistical analyses comparing wild CaSR (WT = wild-type, black) with variants A850V (alone and cotransfected with WT, upper panel), A45E (green), I283T (dar blue), Y161C (blue), C571Y (orange), I826M (pink), Y868D (red), N877D (purple), E765Q+C797W (complex allele, dark pink) and G857V (bordeaux). Responses are expressed relative to the WT response with the mean +/- standard deviation of the mean of the 3-12 replicates.