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CLINICAL STUDY

Identification and functional characterization of loss-of-function mutations of the calcium-sensing receptor in four Italian kindreds with familial hypocalciuric hypercalcemia

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Abstract

Objective: Identification and characterization of calcium-sensing receptor (*CASR*) mutations in four unrelated Italian kindreds with familial hypocalciuric hypercalcemia.

Design: Clinical evaluation and genetic analysis of *CASR* gene. Functional characterization of mutated *CASRs*.

Methods: Direct sequencing of *CASR* gene in genomic DNA. Studies of *CASR*-mediated increases in cytosolic calcium concentration $[Ca^{2+}]_i$ in *CASR*-transfected COS-7 cells *in vitro*.

Results: Four unreported heterozygous *CASR* mutations were identified, including three missense (H595Y, P748H, and C765W) and one splice site (IVS2+1G>C) mutation. The H595Y, P748H, and C765W mutant receptors, although expressed at normal levels on the cell surface, showed a reduced response in $[Ca^{2+}]_i$ relative to the wildtype (WT) *CASR* to increasing extracellular calcium concentrations. Cotransfection experiments showed that the H595Y and P748H mutants did not affect the apparent affinity of the WT *CASR* for calcium, suggesting that they do not exert a dominant-negative effect. On the other hand, the co-transfected C765W mutant decreased the maximum response of the WT *CASR* to calcium, suggesting that it may reduce the effective concentration of the normal *CASR* on the cell surface or impair its maximal signaling capacity.

Conclusions: Four *CASR* mutations were identified. The reduced functional responses to extracellular calcium and normal expression of the mutant receptors suggest that conformational changes account for altered *CASR* activity. Moreover, a reduced complement of normal *CASRs* in these heterozygous patients, perhaps combined with a mutant receptor-induced decrease in maximal activity of the WT receptor, may contribute to defective calcium-sensing *in vivo*.

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Introduction

Familial hypocalciuric hypercalcemia (FHH) is a rare disorder inherited as an autosomal dominant trait with a high penetrance of over 90%, characterized by lifelong, mild to moderate asymptomatic hypercalcemia, relative hypocalciuria, and inappropriately normal PTH levels (1). Patients with FHH exhibit mild to moderate resistance of the parathyroid glands to the inhibitory effects of Ca^{2+} on PTH secretion, resulting in an increase in the 'set-point' of Ca^{2+}-regulated PTH secretion (2).

The cloning of the calcium-sensing receptor (*CASR*) gene has permitted clarification of the molecular basis of FHH. The *CASR* is a G-protein-coupled receptor that senses small perturbations in the level of extracellular Ca^{2+} $[Ca^{2+}]_o$ and modulates the functions of parathyroid and kidney appropriately so as to normalize $[Ca^{2+}]_o$. On the cell surface, the *CASR* forms disulfide-linked dimers through cysteine residues within its extracellular domain and the dimerization has functional implications (3).

Heterozygous loss-of-function mutations, mainly scattered throughout the extracellular domain of the *CASR*, are responsible for most cases of FHH. The majority of mutations are missense (4–6), with a few nonsense (7–9) and splice site mutations (10) as well as an Alu insertion in one family (11, 12). Functional *in vitro* studies have shed light on the mechanisms responsible for the resistance of the parathyroid gland and kidney to calcium. Some mutations produce receptors that do not reach the cell surface and,

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