Mini-review

Activating mutations of the calcium-sensing receptor in primary hypoparathyroidism

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Summary

Familial hypoparathyroidism (also termed autosomal dominant hypocalcemia) is characterized by hypocalcemia, hyperphosphatemia, and relative hypercalciuria, particularly during treatment with vitamin D and calcium to correct the hypocalcemia. Serum parathyroid hormone is inappropriately suppressed. Over 30 activating missense mutations in the calcium-sensing receptor gene have been identified in familial hypoparathyroidism. Functional expression studies *in vitro* show that such mutations typically increase the receptor's sensitivity to Ca^{2+} resulting in suppression of parathyroid hormone secretion and increased urinary calcium excretion at inappropriately low concentrations of serum Ca^{2+}. Identification of calcium-sensing receptor gene mutations in subjects who present with hypocalcemia is important to avoid development of nephrolithiasis during treatment. Activating calcium-sensing receptor gene mutations are non-randomly distributed in the receptor protein. Study of these naturally occurring activating mutations has provided important insights into the structure and function of the receptor.

KEY WORDS: calcium-sensing receptor, activating mutations, familial hypoparathyroidism, autosomal dominant hypocalcemia.

The expression cloning of the bovine calcium-sensing receptor (CaR) just over a decade ago led immediately to studies showing that this novel G protein-coupled receptor (GPCR) is abundantly expressed in parathyroid and kidney where it mediates direct control by serum Ca^{2+} of parathyroid hormone (PTH) secretion and urinary calcium excretion, respectively (1, 2). Increases in serum Ca^{2+} activate the CaR and inhibit PTH secretion from the parathyroid while decreasing reabsorption of filtered calcium in the renal cortical thick ascending limb of Henle. The physiologic importance of the CaR in regulating extracellular Ca^{2+} homeostasis was further emphasized by the discovery of inactivating mutations in the CaR gene as the cause of familial hypocalciuric hypercalcemia (FHH) and neonatal severe hyperparathyroidism, and of activating mutations in the gene as the cause of familial hypoparathyroidism (FH, also termed autosomal dominant hypocalcemia) (3). The phenotypic consequences of loss of CaR function in FHH, hypercalcemia and hypocalciuria, and of gain of CaR function in FH, hypocalcemia and hypercalciuria, are exactly what would be predicted based on the demonstrated function of the CaR in mediating the effects of serum Ca^{2+} in the parathyroid and kidney. In this review, we will focus on FH, and highlight its main genetic, diagnostic, and therapeutic features. We will also discuss how the study of mutations identified in subjects with FH informs our understanding of the structure and function of the CaR.

Familial hypoparathyroidism: discovery of CaR mutations

Familial isolated hypoparathyroidism is a rare disorder which may be inherited in autosomal dominant, recessive, and X-linked forms (4). Mutations in the PTH gene have been identified in both autosomal dominant and recessive forms, and loss of function mutations in the GCMB transcription factor gene have been found in other kindreds with autosomal recessive inheritance (5). However, many cases of isolated hypoparathyroidism remained unexplained until Finegold and colleagues, in a large family with autosomal dominantly inherited isolated hypoparathyroidism, identified linkage to chromosome 3q13, the region harboring the CaR gene (6). Subsequently, Pollak et al described the first missense mutation in the CaR gene in a family with autosomal dominant hypoparathyroidism (7). The activating nature of the missense mutation they identified, an increase in sensitivity ("left-shift" in dose-response) of the mutated receptor to activation by Ca^{2+} compared with the wild type receptor, provided a mechanism for the dominantly inherited phenotype. An activating mutation of a single allele of the CaR gene by conferring increased sensitivity to activation by Ca^{2+} led to suppression of PTH secretion at inappropriately low serum Ca^{2+}, thus resulting in hypocalcemia. Further study of kindreds with activating CaR mutations also showed a tendency to hypercalciuria presumptively due to inhibition of calcium reabsorption in the kidney by inappropriately low serum Ca^{2+} concentration (8). To date, over thirty different activating mutations have been identified in the CaR gene in subjects with FH (9). Several of these have been identified multiple times in apparently unrelated kindreds and individuals. The activating mutations are illustrated in a schematic diagram of the human CaR in Figure 1.

Familial hypoparathyroidism: genetic, clinical and diagnostic features

While hypocalcemia and relative hypercalciuria are the hallmarks of FH caused by activating CaR mutation, the age of onset and severity of symptoms vary widely between different families and even within a family with the identical mutation. In some subjects, presentation in the form of seizures may occur

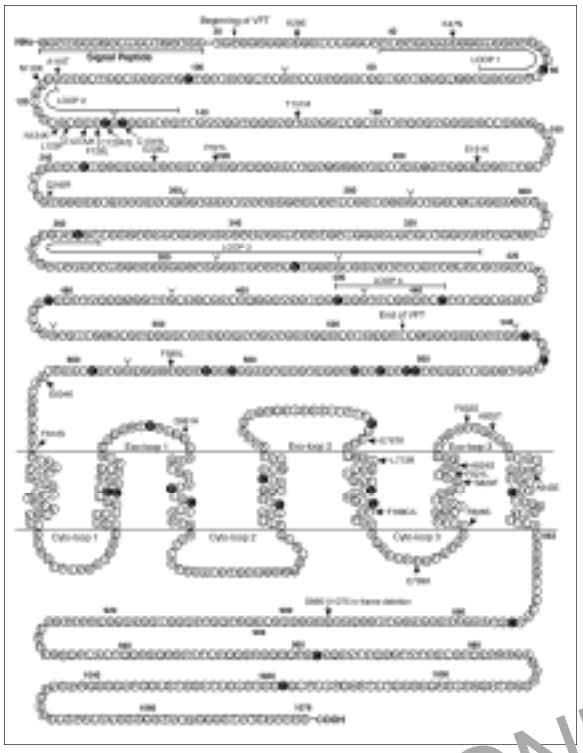

Figure 1 - The amino acid sequence of the human calcium-sensing receptor (single letter code): cysteines (black), N-linked glycosylation sites, signal peptide, beginning and end of venus flytrap domain, loops 1-4 of lobe 1 of the venus flytrap domain, and activating mutations (arrows) identified in subjects with familial hypoparathyroidism are highlighted.

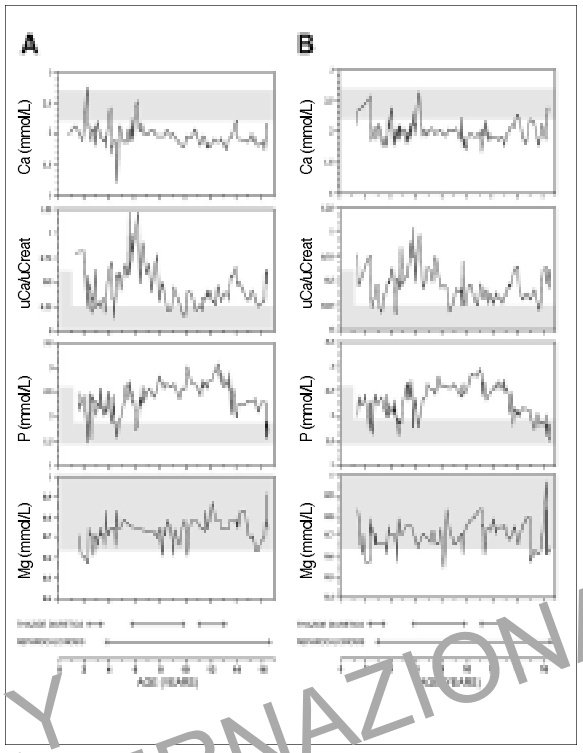

Figure 2 - Concomitant changes of serum calcium, phosphate and magnesium and urinary calcium excretion of monozygotic twins in whom K29E mutation of the calcium-sensing receptor was identified during treatment with oral calcium and dihydrotachysterol (from 2nd postnatal week till age 20 months) or 1,25-dihydroxyvitamin D (from age 20 months onward). Appearance of nephrocalcinosis, and treatment with thiazide diuretics are shown. The shaded areas indicate normal ranges. (reproduced from Hu et al., JBMR 2004; 19:578-586)

shortly after birth (see for example 10), while in others, symptoms of hypocalcemia do not appear until adulthood, or not at all. In one of the largest studies to date, severity of symptoms was independent of age or mutation type but correlated with the degree of hypocalcemia (11). Subjects generally show hyperphosphatemia, and there may be a tendency to hypomagnesemia, consistent with a role for the CaR in regulation of extracellular Mg^{2+} homeostasis (12). Serum intact PTH may be undetectable or within the normal range, but is inappropriately low for the concomitant hypocalcemia. Urinary calcium excretion is higher than with other causes of hypoparathyroidism, and this becomes exacerbated with treatment such as vitamin D and calcium supplements to correct hypocalcemia. This leads to a risk of nephrolithiasis, nephrocalcinosis and renal damage (see Fig. 2). For this reason, some have argued that subjects with FH who are asymptomatic should not be treated. For those in whom symptoms of hypocalcemia demand treatment, vitamin D must be administered cautiously with careful monitoring of urinary calcium excretion. Thiazide diuretics have been advocated to allow reduction in dose of vitamin D for correction of hypocalcemia and simultaneous reduction in hypercalciuria (13). In all cases the aim of treatment is to keep serum calcium concentration at the lower limit of normal, to prevent hypocalcemia-related symptoms. Synthetic 1-34 PTH has also been used to treat subjects with FH, and appeared to be effective in correcting hypocalcemia without inducing hypercalciuria (14). In essence, PTH replacement therapy bypasses the inappropriate inhibition of PTH secretion caused by the activating CaR mutation, but this form of therapy currently requires multiple injections.

While a clear family history and autosomal dominant pattern of inheritance are often found in subjects with FH caused by CaR activating mutation, the disease appears to occur sporadically in some individuals (15). This may be due to *de novo* germline mutations (10, 15), but in at least one family, there was evidence of germline mosaicism for an activating mutation of the CaR gene (16). In this family, both parents were asymptomatic and biochemically normal, but there were two affected siblings with the same activating CaR mutation, and the mother was found to be a mosaic for the same mutation. It is important to distinguish germline mosaicism from a true *de novo* mutation, because of the different risk of having affected offspring in subsequent pregnancies. Because there may not always be a positive family history, some have argued for screening for CaR mutations in all subjects with isolated hypoparathyroidism. Indeed, activating CaR mutations were identified in eight of nineteen probands with isolated hypoparathyroidism in one study (11). Specific diagnosis of activating CaR mutation is important because of the danger of hypercalciuria and nephrolithiasis if hypocalcemia is too vigorously corrected in FH.

In general, no clear pattern of genotype-phenotype correlation has emerged in terms of type or location of CaR mutation, but some suggestive differences have emerged (9). Functional expression studies of various CaR mutations *in vitro* have

demonstrated quantitative differences in the degree of activation in terms of effective concentration 50% (EC_{50}) for Ca^{2+}. Certain mutations such as L125P that have been shown to be particularly "left-shifted" in EC_{50} for Ca^{2+} were identified in subjects with early onset and severely symptomatic hypocalcemia, and the mutation was found to occur *de novo*. This suggests that there may be selection against such powerfully activating mutations, and that they are less likely to occur on a familial basis. A further example is the A843E mutation which was also identified as a *de novo* mutation in a severely symptomatic neonate (11). This mutation is unique in showing a significant degree of constitutive activation in the absence of extracellular Ca^{2+} in *in vitro* expression studies (17). Interestingly, there have been several reports of a Bartter-like syndrome with salt-wasting, hypokalemic metabolic alkalosis, and hyperaldosteronemia, in subjects with FH and activating CaR mutations (18, 19). Since there is evidence that CaR activation can inhibit a renal outer medullary potassium channel, mutations of which result in type 2 Bartter's syndrome, it has been suggested that features of this syndrome may be found in subjects with FH caused by CaR mutations that are particularly activated. Consistent with this hypothesis, mutations identified in such subjects were L125P, A843E, and C131W, all among the most powerfully activating CaR mutations identified (18, 19). The CaR is expressed, albeit at lower levels, in many cells besides the parathyroid and kidney, including brain, pancreatic islet and gastrointestinal tract, and a variety of possible physiologic functions beyond maintenance of extracellular Ca^{2+} homeostasis have been suggested (20). Nonetheless, subjects with germline activating CaR mutations have not demonstrated clinical features that might be due to inappropriate CaR activation in organs other than parathyroid and kidney. Of course, it remains possible that more subtle defects may exist and further studies along these lines are warranted.

Structure and function of the CaR: implications of activating mutations identified in FH

The CaR is a member of family 3 of the GPCR superfamily (9). Family 3 is characterized by a large extracellular amino-terminus consisting of two domains, a bilobed venus flytrap (VFT) domain and a cysteine-rich domain, a seven-transmembrane domain (7TM) typical of all GPCR, and a large intracellular carboxy-terminus (see Fig. 1). Family 3 GPCR exist as dimers, and the CaR is an intermolecular disulfide-linked homodimer in which C^{129} and $C^{131}$are the residues involved in intermolecular disulfide formation (9). X-ray crystallographic studies of the closely related family 3 member, the metabotropic glutamate receptor type 1, have directly demonstrated the dimeric nature of the VFT domain (21). Furthermore, they have shown that agonist binding occurs in the cleft between lobes 1 and 2 of the bilobed VFT, and that such binding leads to a conformational change involving not only closure of the VFT but also a 70° rotation of one monomer relative to the other. In a manner as yet unclear, this agonist-induced rotation of the VFT dimer is transmitted via the cysteine-rich domain to the 7TM domain, causing a conformational change in the latter that leads to activation and G protein coupling.

The location and nature of the activating mutations identified in subjects with FH provide insights into the mechanism of CaR activation. First, the distribution of activating mutations is not random. Figure 1 depicts 32 missense mutations and one in-frame deletion. Only the latter occurs in the intracellular carboxy-terminus. This mutation appears to activate by enhancing receptor expression at the cell surface (11). The missense mutations cluster in three parts of the receptor: thirteen are found in the 7TM, sixteen in the proximal portion of the VFT and three in a region that links the cysteine-rich domain to the 7TM domain. The activating mutations in the VFT appear in a homology model of the CaR VFT based on the crystal structure of the metabotropic glutamate receptor type 1 to cluster at the dimer interface (9). This is particularly notable for a cluster of mutations in loop 2 which is the site of intermolecular disulfide linkage of the VFT dimer. We have suggested that such VFT mutations activate by facilitating the dimer rotation ordinarily engendered by agonist binding (22). In the case of the three mutations in the linker region, it is tempting to speculate that this linker ordinarily is constrained in an inhibitory conformation that is relieved by agonist binding. Activating mutations such as E604K then would mimic agonist binding by relieving this inhibitory constraint. Mutations in the 7TM domain presumptively facilitate conformational changes ordinarily transmitted from the VFT as a consequence of agonist binding. In this regard, the A843E mutation discussed above as unique in causing a degree of constitutive activation, may operate by itself to promote the conformation that leads to G protein coupling and activation. Studies of a form of the CaR harboring the A843E mutation, but in which the entire extracellular domain is deleted, show that it is still constitutively activated, consistent with this hypothesis (17).

Clearly, the identification of activating mutations in the CaR as the cause of FH has had important implications for diagnosis and treatment of this disorder and for our understanding of CaR structure and function. As we learn still more about the precise structure and mechanism of activation of the CaR, we can expect more targeted and effective forms of therapy for subjects with FH.

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