Mini Review

Calcium-sensing receptor: Role in health and disease

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ABSTRACT

The calcium-sensing receptor (CaSR) is a 1,078 amino acid G protein-coupled receptor (GPCR), which is predominantly expressed in the parathyroids and kidney. The CaSR allows regulation of parathyroid hormone (PTH) secretion and renal tubular calcium re-absorption in response to alterations in extracellular calcium concentrations. Loss-of-function CaSR mutations have been reported in the hypercalcemic disorders of familial benign (hypocalciuric) hypercalcemia (FBH or FHH), neonatal severe primary hyperparathyroidism (NSHPT), and adult primary hyperparathyroidism. However, some individuals with loss-of-function CaSR mutations remain normocalcemic. Gain-of-function CaSR mutations have been shown to result in autosomal-dominant hypocalcemia with hypercalciuria (ADHH) and Bartter's syndrome type V. CaSR auto-antibodies have been found in FHH patients who did not have loss-of-function CaSR mutations and in patients with an acquired form (i.e. autoimmune) of hypoparathyroidism. Thus, abnormalities of the CaSR are associated with 4 hypercalcemic and 3 hypocalcemic disorders.

Key words: Hypercalcemia, hypocalcemia, G-protein-coupled receptor

Introduction

The extracellular calcium-sensing receptor (CaSR) is a G-protein-coupled receptor (GPCR) that is predominantly expressed in the parathyroids and kidneys, where it allows regulation of parathyroid hormone (PTH) secretion and renal tubular calcium re-absorption appropriate to the prevailing extracellular calcium concentration. The CaSR is also expressed in other tissues that include the thyroid, intestine, bone, bone marrow, brain, skin, lens epithelium, pancreas, lung and heart, where its function remains to be defined.[1] Ligand binding by the CaSR results in G-protein-dependent stimulation, via Gq/11, of phospholipase C (PLC) activity causing an accumulation of inositol 1,4,5 – trisphosphate (IP_3), and rapid release of calcium ions from intracellular stores $[Ca^{++}]_i$ is followed by an influx of extracellular calcium ions $[Ca{+}{+}]_0$. The increase in $[Ca^{++}]_i$ results in activation of protein kinase C (PKC), which in turn activates the mitogen-activated protein kinase (MAPK) pathway. The CaSR can also activate the MAPK pathway via an isoform of Gi that inhibits adenylate cyclase and that activates Src-family tyrosine kinases. Much has been learnt about the key role of the CaSR in the regulation of extracellular calcium homeostasis by the identification of CaSR mutations in human disorders [Table 1]. Thus, inactivating CaSR mutations result in familial (benign) hypocalciuric hypercalcemia (FBHH), neonatal severe primary hyperparathyroidism (NSHPT), and adult primary hyperparathyroidism (AHPT); whilst activating CaSR mutations result in autosomal-dominant hypocalcemia with hypercalciuria (ADHH), which may be sometimes associated with Bartter-like syndrome.[2-4] Furthermore, CaSR auto-antibodies may also be associated with hypercalcemia or hypocalcemia.

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Hypercalcemic Disorders

CaSR abnormalities are associated with 4 hypercalcemic disorders, which are familial benign hypercalcemia (FBH),

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neonatal severe primary hyperparathyroidism (NSHPT), adult primary hyperparathyroidism, and autoimmune hypocalciuric hypercalcemia (AHH) [Table 1].[5]

Familial benign hypercalcemia

FBH, which is also referred to as familial hypocalciuric hypercalcemia (FHH), is an autosomal-dominant disorder characterized by lifelong and generally asymptomatic hypercalcemia. The hypercalcemia is usually mild to moderate i.e. within 10% of the upper limit of normal, although some patients do have more severe hypercalcemia. Other biochemical features include mild hypermagnesemia, normal or mildly elevated serum PTH concentrations, and an inappropriately low urinary calcium excretion (calcium clearance to creatinine clearance ratio < 0.01). The serum concentrations of 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D are normal, and intestinal calcium absorption may be slightly lower or normal. The disorder is considered to be benign, as patients with FBH are usually asymptomatic. However, there is an increased prevalence of chondrocalcinosis with advancing age and occasional cases of acute pancreatitis and gallstones that have been reported. These symptomatic findings are uncommon in FBH patients who do not require treatment in the form of parathyroidectomy for their hypercalcemia.

Mutational analyzes of the human CaSR gene, located on chromosome 3q21.1, in patients with FBH has revealed different mutations that result in a loss-of-function. Many of these mutations cluster around the aspartate and glutamate rich regions (codons 39 to 300), within the extracellular domain of the receptor, and this has been proposed to contain low affinity calcium-binding sites, based on similarities to that of calsequestrin, in which the ligand-binding pockets also contain negativity charged amino acids. Approximately 65% of the FBH kindreds investigated have been found to have unique heterozygous mutations of the CaSR. Expression studies of the FBH-associated CaSR mutations have demonstrated a loss-of-function, whereby there is a rightward shift in the dose-response curve, such that the extracellular calcium concentration needed to produce a half-maximal (EC_{50}) increase in the total intracellular calcium ions (or IP_3), is significantly higher than that required for the wild-type receptor. This would then result in an increase in the calcium ion-dependent set-point for PTH release from the parathyroid cell. The remaining 35% of FBH families in whom a CaSR mutation has not been detected may either have autoimmune hypocalciuric hypercalcemia, AHH (see below), or an abnormality at 1 of the 2 other FBH loci, which are located on chromosome 19p and 19q13.[6]

Table 1: Diseases associated with calcium-sensing receptor (CaSR) abnormalities

CaSR Abnormality and Disease	CaSR Genotype
Loss-of-function CaSR mutation	
Familial benign hypercalcaemia (FBHH)	Heterozygous
Neonatal severe primary hyperparathyroidism (NSHPT)	Heterozygous or Homozygous (mutant)
Adult primary hyperparathyroidism (AHPT)	Heterozygous or Homozygous (mutant)
Gain-of-function CaSR mutation	
Autosomal dominant hypocalcaemic hypercalciuria (ADHH)	Heterozygous
Bartter syndrome type V	Heterozygous
CaSR Auto-antibodies	
Autoimmune hypocalciuric hypercalcaemia (AHH)	Homozygous (normal)
Acquired hypoparathyroidism (AH)	Homozygous (normal)

Neonatal severe primary hyperparathyroidism

NSHPT is a life-threatening disorder characterized by severe neonatal hypercalcemia, failure to thrive, bony undermineralization, multiple fractures, and ribcage deformity. NSHPT was recognized amongst some children born to consanguineous FBH parents and was thus considered to be the homozygous phenotype of FBH. Indeed, NSHPT, occurring in the offspring of consanguineous FBH families, has been shown to be due to homozygous CaSR mutations. However, patients with sporadic NSHPT have been reported to be associated with *de novo* heterozygous CaSR mutations, and all such mutations result in a loss-of-function.[7] However, all these findings suggest that factors other than mutant gene dosage, for example, the degree of set-point abnormality, the bony sensitivity to PTH, and the maternal extracellular calcium concentration, may also play a role in the phenotypic expression of a CaSR mutation in the neonate.

Adult primary hyperparathyroidism

Adult primary hyperparathyroidism (AHPT) may occur as an isolated familial endocrinopathy, which is referred to as familial isolated primary hyperparathyroidism (FIHP). AHPT due to CaSR mutations is rare, and to date, 13 CaSR mutations (12 missense and 1 truncating), which result in a loss-of-function, have been reported. These AHPT patients develop parathyroid tumors (usually adenomas), and those with mutations located within the extracellular domain of the CaSR have been reported to have a more severe hyperparathyroid phenotype that is associated with a less successful outcome following parathyroidectomy.[8,9]

Autoimmune hypocalciuric hypercalcemia

Some patients, who have the clinical features of FHH but not CaSR mutations, may have this autoimmune disorder of AHH. Such patients with AHH also have other autoimmune manifestations, including anti-thyroid

anti-gliadin and anti-endomyseal antibodies. These patients have circulating antibodies to the extracellular domain of the CaSR, and these antibodies stimulate PTH release from dispersed human parathyroid cells, probably by inhibiting the activation of the CaSR by extracellular calcium. Thus, AHH is a condition of extracellular calcium-sensing that should be considered in FHH patients who do not have CaSR mutations.[10]

Hypocalcemic Disorders

CaSR abnormalities are associated with 3 hypocalcemic disorders, which are autosomal-dominant hypocalcemic hypercalciuria (ADHH, Bartter syndrome type V (i.e. ADHH with a Bartter-like syndrome)), and a form of autoimmune hypoparathyroidism (AH) due to CaSR autoantibodies [Table 1].

Autosomal-dominant hypocalcemic hypercalciuria

CaSR mutations that result in a loss-of-function are associated with familial hypocalciuric hypercalcemia, and it was speculated that CaSR mutations that resulted in a gain-of-function may result in the opposite phenotype of hypocalcemia with hypercalciuria. Investigation of kindreds with autosomal-dominant forms of hypocalcemia identified such CaSR mutations. These patients usually have mild hypocalcemia, which is generally asymptomatic, but may, in some patients, be associated with carpopedal spasm and seizures. The serum phosphate concentrations in patients with ADHH are either elevated or in the upper-normal range, and the serum magnesium concentrations are either low or in the low-normal range. These biochemical features of hypocalcemia, hyperphosphatemia, and hypomagnesemia are consistent with hypoparathyroidism and pseudohypoparathyroidism. However, these patients have serum PTH concentrations that are in the low-normal range, thus they are not hypoparathyroid, which would be associated with undetectable serum PTH concentrations, or pseudohypoparathyroid, which would be associated with elevated serum PTH concentrations.[11] These patients were, therefore, classified as having autosomal-dominant hypocalcemia (ADH), and the association of hypercalciuria with this condition leads it to being referred to as autosomal-dominant hypocalcemia with hypercalciuria (ADHH). Treatment with active metabolites of vitamin D to correct the hypocalcemia has been reported to result in marked hypercalciuria, nephrocalcinosis, nephrolithiasis, and renal impairment, which was partially reversible after cessation of the vitamin D treatment. Interestingly, some patients reported polydipsia and polyuria when they were normocalcemic, and this resolved when they became hypocalcemic. Thus, it is important to identify and avoid vitamin D treatment in such ADHH patients and their families whose hypocalcemia is due to a gain-of-function CaSR mutation and not hypoparathyroidism. Almost every ADHH family has its own unique missense heterozygous CaSR mutation, and expression studies of these mutations have demonstrated a gain-of-function, whereby there is a leftward shift in the dose-response curve, such that the extracellular calcium concentration needed to produce a half-maximal (EC_{50}) increase in the total intracellular calcium ions (or IP_3), is significantly lower than that required for the wild-type receptor.

Bartter syndrome type V

Bartter syndrome is a heterozygous group of autosomal-recessive disorders of electrolyte homeostasis characterized by hypokalemic alkalosis, renal salt wasting that may lead to hypotension, hyper-reninemic hyperaldosteronism, increased urinary prostaglandin excretion, and hypercalciuria with nephrocalcinosis. Mutations of several ion transporters and channels have been associated with Bartter syndrome, and 5 types are now recognized. Thus, type 1 is due to mutations involving the bumetamide-sensitive sodium-potassium-chloride co-transporter (NKCC2 or SLC12A2); type II is due to mutations of the outwardly rectifying renal potassium channel (ROMK); type III is due to mutations of the voltage-gated chloride channel (CLC-Kb); type IV is due to mutations of Barttin, which is a beta sub-unit that is required for trafficking of CLC-Kb and CLC-Ka, and this form is also associated with deafness as Barttin, CLC-Ka and CLC-Kb are also expressed in the marginal cells of the scala media of the inner ear that secrete potassium ion-rich endo lymph; and type V is due to activating mutations of the CaSR. Patients with Bartter syndrome type V have the classical features of the syndrome i.e. hypokalemic metabolic alkalosis, hyper-reninemia, and hyperaldosteronism. In addition, they develop hypocalcemia, which may be symptomatic and lead to carpo-pedal spasm, and an elevated fractional excretion of calcium, that may be associated with nephrocalcinosis. Such patients have been reported to have heterozygous gain-of-function CaSR mutations, and *in vitro* functional expression of these mutations not only revealed a leftward shift in the dose-response curve for the receptor, but also showed them to have a much lower Ec_{50} than that found in patients with ADHH.[12] This suggests that the additional features that occur in Bartter syndrome type V, when compared to ADHH, are due to severe gain-of-function mutations of the CaSR.

Autoimmune-acquired hypoparathyroidism

Twenty per cent of patients who have acquired hypoparathyroidism (AH) in association with autoimmune hypothyroidism were found to have auto-antibodies to the extracellular domain of the CaSR.[13] The CaSR auto-antibodies did not persist for long; 72% of patients

who had AH for less than 5 years had detectable CaSR auto-antibodies, whereas only 14% of patients who had AH for more than 5 years had detectable CaSR auto-antibodies. The majority of the patients who had CaSR auto-antibodies were females, a finding that is similar to that found in other auto-antibody-mediated diseases. Indeed, a few AH patients have also had features of autoimmune polyglandular syndrome type 1 (APS1). These findings establish that the CaSR is an auto antigen in AH.[14,15]

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References

1. Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, *et al*. Cloning and characterization of an extracellular Ca2+-sensing receptor from bovine parathyroid. Nature 1993;366:575-80.
2. PollakMR, Brown EM, Chou YH, Hebert SC, Marx SJ, Steinman B, *et al*. Mutations in the human Ca2+ sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell 1993;75:1297-303.
3. Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, *et al*. Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation. Nat Genet 1994;8:303-7.
4. Pollak MR, Chou YH, Marx SJ, Steinmann B, Cole DE, Brandi ML, *et al*. Familial hypocalciuric hypercalcaemia and neonatal severe hyperparathyroidism. Effects of mutant gene dosage on phenotype. J Clin Invest 1994;93:1108-12.
5. Pearce SH, Trump D, Wooding C, Besser GM, Chew SL, Grant DB, *et al*. Calcium-sensing receptor mutations in familial benign hypercalcemia and neonatal hyperparathyroidsm. J Clin Invest 1995;96:2683-92.
6. Kifor O, Moore FD Jr, Delaney M, Garber J, Hendy GN, Batters R, *et al*. A syndrome of hypocalciuric hypercalcaemia caused by auto-antibodies directed at the calcium-sensing receptor. J Clin Endo Metab 2003;88:60-72.
7. Bai M, Pearce SH, Kifor O, Trivedi S, Stanffer UG, Thakker RV, et al. *In vivo* and *in vitro* characterisation of neonatal hyperparathyroidism resulting from a *de novo*, heterozygous mutation in the Ca^{2+}-sensing receptor gene: Normal maternal calcium homeostasis as a cause of secondary hyperparathyroidism in familial benign hypocalciuric hypercalcaemia. J Clin Invest 1999;99:88-96.
8. Brown EM, MacLeod RJ. Extracellular calcium-sensing and extracellular calcium signalling. Physiol Rev 2001;81:239-97.
9. Hannan FM, Nesbit MA, Christie PT, Lissens W, Van der Schueren B, Bex M, et al. A homozygous inactivating calcium-sensing receptor mutation, Pro339Thr, is associated with isolated primary hyperparathyroidism: Correlation between location of mutations and severity of hypercalcaemia. Clin Endocrinol 2010;73: 715-22.
10. Thakker RV. Diseases associated with the extracellular calcium-sensing receptor. Cell Calcium 2004;35:275-82.
11. Pearce SH, Williamson C, Kifor O, Bai M, Coulthard MG, Davies M, *et al*. A familial syndrome of hypocalcaemia with hypercalciuria due to mutations in the calcium-sensing receptor. New Eng J Med 1996;335:1115-22.
12. Watanabe S, Fukumoto S, Chang H, Takenchi Y, Hasegaiva Y, Okazaki R, et al. Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. Lancet 2002;360:692-4.
13. Li Y, Song Y-H, Rais N, Connor E, Schatz D, Muir A, *et al*. Auto antibodies to the extracellular domain of the calcium-sensing receptor in patients with acquired hypoparathyroidism. J Clin Invest 1996;97:910-4.
14. Kemp EH, Gavalas NG, Krohn KJ, Brown WM, Watson PF, Weetman AP. Activating autoantibodies against the calcium-sensing receptor detected in two patients with autoimmune polyendocrine syndrome type 1. J Clin Endocrinol Metab 2009;94:4749-56.
15. Hannan FM, Nesbit MA, Zhang C, Cranston T, Curley AJ, Harding B, *et al*. Identification of 70 calcium-sensing receptor mutations in hyper- and hypo-calcaemic patients: Evidence for clustering of extracelluar domain mutations at calcium-binding sites. Hum Mol Genet 2012;21:2768-78.

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