Published in final edited form as:
Best Pract Res Clin Rheumatol. 2008 March ; 22(1): 129–148. doi:10.1016/j.berh.2007.11.006.

Hypercalcaemic and hypocalcaemic conditions due to calcium-sensing receptor mutations

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Abstract

The extracellular calcium (Ca^{2+}_o)-sensing receptor (CaSR) enables the parathyroid glands and other CaSR-expressing cells involved in calcium homeostasis, such as the kidney and bone, to sense alterations in the level of Ca^{2+}_o and to respond with changes in function that are directed at normalizing the blood calcium concentration. Several disorders of Ca^{2+}_o sensing arise from inherited or acquired abnormalities that 'reset' the serum calcium concentration upwards or downwards. Heterozygous inactivating mutations of the CaSR produce a benign form of hypercalcaemia, termed 'familial hypocalciuric hypercalcaemia', while homozygous mutations produce a much more severe hypercalcaemic disorder resulting from marked hyperparathyroidism, called 'neonatal severe hyperparathyroidism'. Activating mutations cause a hypocalcaemic syndrome of varying severity, termed 'autosomal-dominant hypocalcaemia or hypoparathyroidism' as well as Bartter's syndrome type V. Calcimimetic CaSR activators and calcilytic CaSR antagonists have also been developed with potential for use in the treatment of these disorders.

Keywords

seven transmembrane receptor; mutations; polymorphisms; calcium-sensing receptor; calcium homeostasis; calcimimetic; calcilytic; familial hypocalciuric hypercalcaemia; autosomal-dominant hypoparathyroidism; acquired hypoparathyroidism; osteoporosis; hyperparathyroidism; Bartter's syndrome

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The calcium-sensing receptor (CaSR) plays key roles in the maintenance of a narrow range (1.1–1.3 mM) of the extracellular ionized calcium concentration (Ca^{2+}_o), primarily by modulating the function of chief cells of the parathyroid gland. Here it regulates the synthesis and secretion of parathyroid hormone (PTH) as well as parathyroid cellular proliferation, inhibiting all three when Ca^{2+}_o is high and stimulating them when Ca^{2+}_o is low.[1] Both very high and very low levels of Ca^{2+}_o can lead to serious clinical sequellae and, in some instances,

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can be life-threatening. The CaSR serves as a 'calciostat', informing the parathyroid glands and other tissues of the precise level of Ca^{2+}_o.

The CaSR (also known as CaSR1 and GPRC2A) was cloned using the expression-cloning technique in *Xenopus laevis* oocytes.[2] It is a member of Family C of the superfamily of seven transmembrane (7TM), G-protein-coupled receptors (GPCRs). Other members of this family are so-called metabotropic receptors for glutamate, receptors for gamma-aminobutyric acid, and GPCRs for sensing pheromones, taste and odorants (in fish). Recently, another member of Family C, GPRC6A, has been found to share several pharmacological properties with the CaSR.[3,4] Like the CaSR, GPRC6A is sensitive towards certain L-amino acids, but is most responsive to basic amino acids and less so to calcium,[3,5] implicating GPRC6A as a second calcium-sensing receptor or at least a calcium-modulated amino acid sensor.

The physiological relevance of the CaSR in humans was proven by the identification of inherited disorders caused by mutations in the receptor leading to either loss or gain of function.[6] Heterozygous gain-of-function mutations (CaSR activating) or loss-of-function mutations (CaSR inactivating) are the cause of a growing number of disorders of calcium metabolism, which typically manifest as asymptomatic hypo- or hypercalcaemia, respectively, with relative or absolute hyper- or hypocalciuria. When present in the homozygous or compound heterozygous state, in contrast, inactivating CaSR mutations produce neonatal severe primary hyperparathyroidism (NSHPT), a severe and sometimes lethal disease if it is left untreated.

CaSR expression is greatest in the parathyroid glands, calcitonin-secreting C-cells of the thyroid gland, and kidney. The CaSR is also found in the two other key organs that participate in calcium homeostasis: gut and bone (Figure 1).[7,8] This review will briefly discuss the structure, function and normal physiology of the CaSR, and discuss in more depth the mutations and polymorphisms of the CaSR, and its role in various disorders of Ca^{2+}_o sensing that are the result of mutations in the CaSR.

PHYSIOLOGY OF THE CASR

PTH, calcitonin and 1,25-dihydroxyvitamin D [$1,25(OH)_2D_3$] are the three most important Ca^{2+}_o-regulating hormones.[9] As noted earlier, there is a functionally critical inverse relationship between Ca^{2+}_o and PTH, a calcium-elevating hormone. In contrast, high Ca^{2+}_o stimulates the secretion of calcitonin, a Ca^{2+}_o-lowering hormone; an action that is likewise mediated by the CaSR.[10] Available data have demonstrated that the CaSR is expressed not only in the organs that secrete calcium-regulating hormones (e.g. the parathyroid glands and C-cells of the thyroid glands), but also in target tissues for these hormones. These latter tissues regulate Ca^{2+}_o by translocating calcium ions into or out of the bodily fluids, and include the kidney, which expresses the CaSR at robust levels in certain nephron segments, and bone and intestine, which express the receptor at lower levels (Figure 1).[7] By acting on both hormone-secreting and hormone-responsive tissues through its own cell surface receptor, Ca^{2+}_o acts, in effect, as another Ca^{2+}_o-regulating 'hormone' (in this case, Ca^{2+}_o lowering) or 'first messenger'. Elevations in Ca^{2+}_o stimulate the CaSR and lower Ca^{2+}_o by enhancing calcitonin secretion, promoting urinary calcium excretion and suppressing PTH release. The remainder of this section will provide a brief discussion of the CaSR's known and putative roles in parathyroid gland, kidney and bone; three of the four main organs involved in calcium homeostasis. A recent review discusses the CaSR's localization and potential roles along the gastrointestinal tract.[11]

Parathyroid glands

The CaSR is normally expressed at high levels on the surface of the parathyroid chief cells, where it mediates its most important function in minute-to-minute Ca^{2+}_o homeostasis by CaSR-

regulated inhibition of PTH secretion. The steep inverse sigmoidal curve relating Ca^{2+}_o and PTH release was described long before the CaSR was cloned.[12]

The principal functions of the CaSR in the parathyroid gland are shown in Table 1. Relatively little is known about the control of its expression on the cell surface, but of note, CaSR expression in rat parathyroid and kidney was increased by $1,25(OH)_2D_3$, while Ca^{2+}_o had no effect.[13] In more recent studies, however, raising Ca^{2+}_o increased CaSR expression in avian parathyroid gland,[14] and a calcimimetic elevated CaSR expression in pathological parathyroid glands.[15] The upregulation of CaSR following its activation could clearly serve as a positive feedback loop contributing to CaSR-mediated actions in the parathyroid gland. Of note in this regard, Ca^{2+}_o also regulates the expression of the vitamin D receptor,[16] which could potentiate the action of vitamin D on the level of CaSR expression and also enhance the biological actions of the CaSR.

Expression, function and regulation of the CaSR in the kidney

The kidney plays several critical roles in calcium homeostasis. The CaSR is widely expressed along essentially the whole nephron. The cellular localization and putative function(s) of the CaSR in the kidney seem to depend upon the region of the nephron in which the receptor resides.[17] The CaSR's cellular localization and expression along the nephron has been studied using in-situ hybridization, reverse transcriptase-polymerase chain reaction of micro-dissected nephron segments[18] and immunofluorescence.[19] One outcome of these studies was the recognition that the polarity of CaSR protein varies along the nephron. In the proximal tubule, the receptor is present on the apical surface of the proximal tubular epithelial cells. On the contrary, in the cells of the cortical thick ascending limb (CTAL), the receptor is localized in the basolateral membrane. Similarly, basolateral staining for the CaSR was observed in the medullary thick ascending limb, macula densa and the distal convoluted tubule. In the cortical collecting duct, immunostaining for the CaSR is located on some intercalated cells, while in the inner medullary collecting duct (IMCD), the receptor has primarily an apical distribution.

Available data support the following roles of the CaSR along the nephron: (1) diminishing the inhibitory effect of PTH on renal phosphate re-absorption in the proximal tubule;[20] (2) inhibiting renal tubular re-absorption of calcium in the CTAL;[21] and (3) reducing urinary concentrating ability in the IMCD by antagonizing the action of vasopressin.[22]

There has been relatively little work characterizing the factors that regulate CaSR expression in the kidney. A recent report demonstrated that in rat kidney, C-cell and parathyroid in vivo, as well as in a human proximal tubule cell line in vitro, transcription of the *CaSR* gene was significantly increased following 8 and 12 h of treatment with $1,25(OH)_2D_3$,[23] acting via vitamin D response elements upstream of the *CaSR* gene. Riccardi et al demonstrated in vivo in rats that a low phosphate diet as well as treatment with PTH downregulated CaSR protein in the proximal tubule.[24] Thus, CaSR expression in the proximal tubule of the rat kidney is modulated by $1,25(OH)_2D_3$, PTH and, perhaps, dietary phosphate.

Bone

Abundant data indicate that Ca^{2+}_o inhibits the formation and activity of osteoclasts and stimulates the activity of osteoblasts. The first evidence for the existence of a G-protein-coupled, cation-sensing mechanism in osteoblasts was presented shortly after the cloning of the CaSR.[25] Since then, some, but not all, studies have found that the CaSR is expressed in various osteoblastic cell lines and primary osteoblasts.[26,27] An interesting study demonstrated that osteoblasts from CaSR knockout mice still had a promitogenic response to Ca^{2+}_o, supporting the presence of a calcium-sensing mechanism other than the full-length CaSR. This mechanism could potentially be represented by the newly cloned GPRC6A, although the latter

is responsive to calcimimetics, and the actions of Ca^{2+}_o on the CaSR knockout osteoblasts were not.[5] The CaSR is also present in articular and hypertrophic chondrocytes.[26] Utilizing a type II CaSR agonist in organ culture (fetal rat metatarsal bones) to study the possible role of the CasR in bone growth, Wu et al[28] demonstrated that the receptor modulates chondrogenesis in the growth plate and enhances longitudinal bone growth.

The CaSR is expressed by some osteoclasts[29] and by monocytes, which are of the same lineage as osteoclast precursors.[30] In addition to inhibiting the formation and activity of osteoclasts, high Ca^{2+}_o has been shown to promote osteoclast apoptosis.[31] However, the calcimimetic, AMG 073, produced none of the actions of elevated Ca^{2+}_o on osteoblast proliferation or osteoclast formation and resorption in one study.[32] One possible mechanism that has been suggested to mediate calcium sensing in osteoclasts is a plasma membrane, ryanodine-like receptor that couples to increases in the intracellular calcium concentration.[33] Therefore, although the CaSR and other calcium-sensing mechanisms may participate in the regulation of bone cell and cartilage function, further studies are clearly required to clarify the divergent results observed in studies to date.

MOLECULAR BIOLOGY OF THE CASR

This section briefly introduces key aspects of the structure and function of the CaSR to provide sufficient background information to understand the molecular basis for both normal mineral ion homeostasis and diseases from mutations of the CaSR.

Structure and signalling pathways of the CaSR

The 5.3-kb clone of the CaSR isolated by expression cloning, when expressed in oocytes, exhibited the same pharmacological properties as the Ca^{2+}_o-sensing mechanism previously characterized in dispersed bovine parathyroid cells, the prototypical calcium-sensing cell.[2] The use of nucleic-acid-hybridization-based cloning enabled the CaSR to be cloned from humans[34] and several other animals. The nucleic acid sequences of the mammalian receptors are at least 85% identical to that of the original bovine parathyroid CaSR. The amino acid sequences show even greater similarity (>90% identity using http://www.ncbi.nlm.nih.gov/BLAST/). Therefore, only a limited amount of divergence from a putative primordial calcium-sensing receptor has taken place throughout evolution, and the functionally important structural features have presumably been retained.

The CaSR is a member of Family C II of the superfamily of 7TM receptors, also called ‘GPCRs’,[8] which is by far the largest group of cell surface receptors. They are critical in clinical medicine, since the 7TM receptors represent the targets of about 50% of currently available drugs. The human CaSR comprises 1078 amino acid residues and has three structural domains, as do all 7TM receptors (Figure 2). It has a large extracellular domain (ECD) (612 residues), a transmembrane domain (TMD) of 250 amino acids containing the seven membrane-spanning helices, and an intracellular, C-terminal domain (ICD) of 216 amino acids. The receptor exhibits substantial N-linked glycosylation, which is important for normal cell membrane expression of the receptor, but does not appear to modify the function of the receptor per se.[35] The functional cell surface form of the CaSR is a dimer, and the two monomers within the dimeric CaSR are linked by disulphide bonds involving cysteine residues 129 and 131 within each monomer.[36] The ECD of each CaSR monomer probably contains more than one binding site for Ca^{2+}_o because the Hill coefficient for the activation of the receptor by Ca^{2+}_o is 3–4, consistent with the presence of positive cooperativity amongst at least this number of binding sites within the dimeric CaSR.[37,38] The TMD also appears to be involved in Ca^{2+}_o sensing, since a mutant CaSR lacking the ECD also responds to Ca^{2+}_o and other polyvalent cations.[39]

The CaSR is located in caveolin-1-rich membrane domains called 'caveolae' from which it initiates several signalling pathways upon activation in cooperation with several other signalling molecules such as filamin-A and caveolin-1. The CaSR, through $G\alpha_{q/11}$ or $G\alpha_{i/0}$, activates PLA_2, PLD, PLC, PKC, PI3K and mitogen-activated protein kinases like ERK-1/2, but the critical pathway(s) through which the CaSR mediates its biological effects is(are) yet to be identified. A detailed description of CaSR-mediated signalling is beyond the scope of this review, but the reader is referred to a recent discussion of this topic.[40]

Agonists and antagonists of the CaSR

The CaSR behaves in a promiscuous manner, as there are a considerable number of ligands that modulate its function. CaSR agonists are described as type I or type II.[41] Type I agonists are direct agonists listed here in order of potency $Gd^{3+} \geq La^{3+} \gg Ca^{2+} = Ba^{2+} > Sr^{2+} > Mg^{2+}$,[42] while type II agonists serve as allosteric modulators, requiring the presence of calcium to stimulate the CaSR. Type II modulators left-shift the calcium dose–response curve by sensitizing the receptor to type I agonists and include small molecule drugs and amino acids. The best characterized type I organic polycationic CaSR agonists are gadolinium, neomycin, spermine and amyloid β-peptides.[2,43] Newer, more specific approaches, such as the use of pharmacological activators or inhibitors of the receptor, dominant negative constructs or RNA silencing, offer better opportunities for specifying the functional activity of the receptor.

Drugs that allosterically stimulate the CaSR are called 'calcimimetics'. NPS R-467, NPS R-568 and AMG 073 are calcimimetics that have been used in various experimental studies and clinical trials and, more recently, as treatments for secondary hyperparathyroidism. AMG 073 (also called cinacalcet hydrochloride, Sensipar or Mimpara) is currently the drug of choice, because NPS R-467 and NPS R-568 are degraded by a cytochrome P-450 enzyme, CYP2D6 (Amgen, unpublished data). Five to seven percent of the general population express CYP2D6, which has reduced enzymatic activity, thereby resulting in higher blood levels and delayed metabolic clearance in this segment of the population. Calcimimetics interact with the TMD of the CaSR and increase the affinity of the receptor for calcium. Some L-amino acids also act as type II agonists, in contrast to the respective D-amino acids which are several-fold less potent in activating the receptor.[44] Finally, calcilytics represent another type of pharmacological agent acting on the CaSR. They antagonize the action of Ca^{2+}_o on the receptor and are being studied for use in the treatment of osteoporosis, as they stimulate a pulse of endogenous PTH secretion which has the potential to exert an anabolic action on bone, similar to that of once-daily injected PTH.[45]

DISORDERS OF CALCIUM SENSING THAT INVOLVE CASR MUTATIONS

The principal disorders of Ca^{2+}_o sensing are listed in Table 2.

Clinical and genetic features of familial hypocalciuric hypercalcaemia (OMIM 14598)

The characteristic clinical features of the relatively benign condition now known as familial hypocalciuric hypercalcaemia (FHH) (initially called 'familial benign hypercalcaemia') were first descrided in 1972. The diagnosis of FHH can be made in a patient with mild-to-moderate, PTH-dependent hypercalcaemia averaging approximately 2.75 mM (total calcium), an autosomal-dominant pattern of inheritance of a similar degree of hypercalcaemia on family screening, and an inappropriately reduced rate of urinary calcium excretion in the face of hypercalcaemia. Several families, however, have been identified with more marked hypercalcaemia, averaging 3 and 3.4 mM. Patients with FHH are frequently not diagnosed until a routine measurement of the blood calcium concentration shows an unexpectedly high value, or family screening is carried out owing to the birth of a child with NSHPT. Patients

with FHH commonly have normal serum levels of PTH despite their hypercalcaemia, although in approximately 15–20% of cases, PTH levels are frankly elevated.[46]

The hypercalcaemia in FHH, with PTH levels inappropriately high for that serum calcium concentration, reflects the presence of a right-shifted set-point for Ca^{2+}_{o}-regulated PTH release.[47] An additional important finding is the normal to frankly reduced urinary calcium excretion in spite of the coexistent hypercalcaemia.[48] This alteration in renal calcium handling reflects 'resistance' of the kidney to the usual hypercalciuric action of hypercalcaemia, and is the equivalent in the kidney of the resistance of PTH secretion in the parathyroid gland to the normal inhibitory effect of high calcium. Of note, administration of a loop diuretic (e.g. ethacrynic acid) promotes renal excretion of calcium in hypoparathyroid subjects with FHH,[49] and more recently has been observed in CaSR knockout mouse models (unpublished observations). These observations point towards a key role of the thick ascending limb (the principal site of action for these classes of diuretics) in the anomalous renal calcium handling in FHH.

Short of carrying out mutational analysis, the most useful means of distinguishing FHH from other forms of hypercalcaemia, particularly primary hyperparathyroidism, is to determine the ratio of the renal clearance of calcium to that of creatinine. A value less than 0.01 is found in approximately 80% of individuals with FHH, while a similar proportion of cases of primary hyperparathyroidism have levels higher than this.[50] Another biochemical finding in patients with FHH is their capacity to concentrate their urine normally, in contrast to patients with primary hyperparathyroidism in whom maximal urinary concentration elicited by dehydration is reduced.[51] While this finding is not used diagnostically, it reflects renal resistance of FHH patients to the hypercalcaemia-induced diminution in urinary concentrating ability that is observed in other forms of hypercalcaemia. Individuals with FHH usually manifest serum magnesium concentrations that are in the upper normal range or mildly elevated.

While differentiating FHH from primary hyperparathyroidism is usually straightforward, a study of the genetic basis for familial isolated hyperparathyroidism showed that four of 22 unrelated probands harboured inactivating mutations of the CaSR[52] restricted to the parathyroid gland. Therefore, the clinician should bear in mind that FHH can be an underdiagnosed but important cause of familial isolated hyperparathyroidism.[52,53] Moreover, this study points out that there can be overlap in the clinical presentations of FHH and primary hyperparathyroidism that are important treatment considerations, particularly in familial forms of the latter. This is aptly exemplified by an atypical presentation in an FHH family that exhibited hypercalcaemia, high PTH levels, hypercalciuria and even renal stone formation, but was ultimately proven to harbour an inactivating FHH mutation.[54] Subtotal parathyroidectomy in most affected family members provided long-term remission of their biochemical abnormalities, demonstrating that parathyroid surgery, while typically ineffective in curing hypercalcaemia in FHH, may be appropriate in occasional kindreds. While FHH most commonly presents as an asymptomatic form of hypercalcaemia, a few kindreds exhibit more severe hypercalcaemia. Nevertheless, even in these cases, the natural history of the disorder is usually so benign that the great majority of these patients should be followed without intervention, with a few exceptions (see above). In the unusual individual with FHH and symptomatic hypercalcaemia, the new calcimimetics could potentially provide a useful form of treatment.

Finally, studies have described the presence of single and multiple parathyroid 'adenomas' in several patients with FHH, although the parathyroid glands in FHH are typically of normal size and histology, or in some cases exhibit mild chief cell or lipohyperplasia with one or more enlarged glands resembling adenomas.[54,55]

Two hundred and thirteen mutations have been described for the CaSR (188 mis-sense, 17 non-sense, six insertion and/or deletion, one silent and one splice mutation) in the CaSR mutation database (http://www.casrdb.mcgill.ca) related to FHH, NSHPT or ADH families or as de-novo disease. Of these mutations, most are inactivating with the most frequently reported for FHH being 59 mis-sense mutations, six non-sense mutations, six insertions and/or deletions including an Alu element insertion[56] and one splice mutation.[57] A number of dominant negative mutations (S137P, R185Q, R227L, R795W and F881L) have also been described that appear to inhibit the wild-type partner in mutant wild-type heterodimers, thereby increasing the degree of hypercalcaemia. Linkage analysis showed that the predominant locus of the FHH disease gene (e.g. the *CaSR* gene) resided on the long arm of chromosome 3 (band q21–24).[58] However, FHH is not always linked to chromosome 3q. Notably, two families with clinical features similar to FHH showed linkage to the short and long arms of chromosome 19, respectively; one of these was called the 'Oklahoma variant' and exhibited a tendency for the biochemical abnormalities to progress with time.[59] FHH that is linked to these latter two loci may be present in a minority of the ~30% of FHH cases without an identifiable mutation in the *CaSR* gene. The remaining cases of FHH without an identifiable mutation presumably harbour mutations in regulatory regions of the *CaSR* gene that control its expression, but this remains to be shown directly. Three cases of de-novo NSHPT have been described that were heterozygous for mis-sense mutations located in the ECD, and no mutation was found in the parents.[60,61] One individual with de-novo NSHPT was heterozygous for a previously described mutation in an FHH family.[60]

Clinical and genetic features of neonatal severe primary hyperparathyroidism (OMIM 239200)

There appears to be a gene dosage effect in many inactivating mutations of the CaSR, with heterozygous inactivating mutations leading to FHH with mild hypercalcaemia, and homozygous mutations resulting in NSHPT, a more severe phenotype that manifests very early in life with severe hypercalcaemia, bone demineralization and failure to thrive.

In most cases, NSHPT presents within the first 6 months of life. Affected infants have severe, symptomatic, PTH-dependent hypercalcaemia, along with the bony changes of severe hyperparathyroidism. Infants with NSHPT can exhibit polyuria, dehydration, hypotonia and failure to thrive.[46,62,63] A prominent feature of the disease is the associated hyperparathyroid bone disease, which can be associated with multiple fractures. Rib fractures can, in some cases, produce a 'flail chest' syndrome that causes respiratory difficulties, owing to a decreased capacity of the affected infant to expand its chest wall and generate the negative intrathoracic pressure needed for normal respiration.[64]

The mass of the parathyroid glands in NSHPT is generally increased several fold, and they exhibit prominent chief cell hyperplasia. Biochemical evaluation shows hypercalcaemia, hyperparathyroidism and relative hypocalciuria.[65] Total serum calcium concentrations range from moderately elevated (e.g. 3–3.25 mM) to levels as high as 7.7 mM in the most severely affected cases.[46,66] PTH levels are often 10-fold higher than the upper limit of normal. Early diagnosis is critical as untreated NSHPT can be a devastating neurodevelopmental disorder, which in some cases is lethal without parathyroidectomy to alleviate the hyperparathyroidism and hypercalcaemia.[65] As noted earlier, the most severe cases of NSHPT develop ribcage deformities, as well as rachitic changes, skeletal undermineralization, and fractures of the long bones and other skeletal sites.[64,67]

More recently, a broader clinical spectrum for NSHPT has become apparent, particularly given the availability of genetic testing of the *CaSR* gene. As a result, a number of studies have shown that some infants have milder hyperparathyroidism and a substantially milder clinical presentation and natural history.[46,61] This latter form of the disease might be better termed 'neonatal hyperparathyroidism' to emphasize this milder phenotype in these infants, most of

whom harbour heterozygous inactivating CaSR mutations. In these latter cases, the condition can revert with time to a phenotype resembling FHH with medical management alone.[46] Therefore, at the moment, parathyroidectomy should be reserved for the most severely affected infants in whom intensive medical therapy (e.g. with aggressive hydration and, if appropriate, bisphosphonates) has failed to stabilize the patient, and there is concern for the infant's survival.

Three cases of de-novo NSHTP reported in the literature are heterozygous for mis-sense mutations located in the ECD, with only one mutated allele and no mutation found in the parents.[60,61] One individual with de-novo NSHPT was heterozygous for a previously described mutation in a FHH family,[60] and it is thought that the mutant receptors with these mutations in some cases exert a dominant negative action on the wild-type partner in mutant wild-type heterodimers. Recent reports have described patients with homozygous mutations in the *CaSR* gene who escaped detection until adulthood, at which time they did not have the usual symptoms and signs of hypercalcaemia and were only identified serendipitously by routine biochemical screening. One such patient, a 35-year-old woman, had two copies of the mis-sense mutation pro39ala from related parents. She was asymptomatic, despite a serum calcium concentration of 3.75–4.25 mM.[68] Another such patient, who was homozygous for a distinct inactivating CaSR mutation, was also not diagnosed until adulthood.[69] Both mutations produced relatively mild defects of their function when expressed heterologously, perhaps enabling sufficient control of PTH release by calcium to be compatible with a relatively normal life, despite quite marked hypercalcaemia. Indeed, the seeming lack of hypercalcaemic symptoms in the face of moderate to severe hypercalcaemia supports the notion that at least some of these symptoms are mediated by the CaSR. That is, these patients seem to be resistant not only to the effects of calcium on parathyroid gland and kidney, but also to the development of hypercalcaemic symptoms. In these patients, a calcimimetic might represent a means of lowering the serum calcium concentration, assuming the mutant CaSRs were responsive to the drug, thereby providing not only a diagnostic test to determine whether the patient obtained any symptomatic benefit from parathyroidectomy but also, potentially, an effective long-term medical therapy.

NSHPT is most commonly an autosomal-recessive condition; that is, the *CaSR* genes from both of the parents are mutated (e.g. homozygous FHH). Pollak et al studied 11 kindreds with FHH in whom consanguineous unions engendered four infants with NSHPT.[70] It should be recognized, however, that NSHPT is quite uncommon in FHH families considered as a whole. In one case of NSHPT, two distinct mutations, one a mutation in exon 7 from the mother and the other a mutation in exon 4 from the father, caused the disease as a result of the compound heterozygosity in the proband, who thereby lacked any normal CaSRs.[71] In theory, NSHPT can result from: (1) homozygosity from a consanguineous FHH union; (2) two mutant alleles of the *CaSR* gene arising from two distinct FHH kindreds; or (3) from a de-novo mutational event, with or without an inherited, mutant parental allele.[61] In addition, an investigation of a girl with phenotypic NSHPT and her family revealed a single mutant allele (present in exon 6, Gly552Arg) in her *CaSR* gene, while her sister, despite having the same genotype, had phenotypic FHH.[72] Thus, factors leading to this degree of phenotypic variation are still only partly understood.

Clinical and genetic features of autosomal-dominant hypoparathyroidism (OMIM 601298)

Patients with this inherited form of hypocalcaemia/hypoparathyroidism are commonly asymptomatic. Some patients, especially children during febrile episodes, can exhibit neuromuscular irritability, seizures and basal ganglia calcification. Patients generally exhibit mild to moderate hypocalcaemia, with serum PTH levels that are inappropriately low given the hypocalcaemia, i.e. within the lower half of the normal range or frankly subnormal.[73] Affected individuals often exhibit relative or absolute hypercalciuria, with normal or frankly

elevated urinary calcium excretion, respectively, in spite of their low serum calcium concentration. Some, but not all, studies have shown that renal calcium excretion in ADH is higher than that in typical hypoparathyroidism. It is important to prevent renal complications, including nephrocalcinosis, impaired renal function and nephrolithiasis, during treatment of ADH patients with calcium and vitamin D. Treatment with calcium supplements and vitamin D should be reserved for those patients with symptomatic ADH; the goal should be to increase the serum calcium concentration only to a level sufficient to render the patient asymptomatic and not necessarily to a normocalcaemic level. Renal excretion of calcium requires monitoring, and it may be necessary to co-administer a hypocalciuric agent, such as a thiazide diuretic or injectable PTH,[74] that can lower urinary calcium excretion at any given level of serum calcium.

ADH, although rare, in index cases may comprise a sizeable fraction of cases of idiopathic hypoparathyroidism, perhaps representing as many as one-third of such cases.[75] Patients with this condition harbour an activating mutation of the *CaSR* gene that resets the set-point of Ca^{2+}_o-regulated PTH secretion to the left and lowers renal calcium re-absorption (Figure 2). Soon after the cloning of the CaSR, investigators[76] showed linkage of ADH to a locus on chromosome 3 q13; the same locus containing the gene for the CaSR. Shortly afterwards, a heterozygous mis-sense mutation, Q127A, was shown to be the cause of ADH in an unrelated family.[73] Since these first reports, 40 mutations have been characterized causing ADH. The majority are mis-sense mutations within the CaSR's ECD and TMD. In addition, two deletion mutations have been described. Most ADH patients are heterozygous for the activating mutation. In one family, a homozygous mutation was described but it was not associated with a more severe phenotype,[77] and although there is a spectrum of phenotypic severity for a given genotype, the symptoms present in affected members of the same family tend to be similar.

When expressed in heterologous systems, these mutations cause a left-shift in the activation of the CaSR by Ca^{2+}_o; they only rarely induce constitutive activation of the receptor.[58,73,78–80] A recent report identified a family with a large deletion of 181 amino acids within the C-terminus of the CaSR, which increased the sensitivity of the receptor for Ca^{2+}_o.[77] This family contained the only individual to date known to be homozygous for an activating mutation, but this individual exhibited a phenotype very similar to that of the heterozygous family members. Thus, one mutated allele may be enough to induce a maximal shift in the set-point of Ca^{2+}_o-regulated PTH secretion, and the presence of the second mutated allele does not alter the biochemical properties of the receptor dimers any further, perhaps due to a 'dominant positive' effect of the mutant receptor on its wild-type partner within the heterodimeric CaSR. Another activating mutation of the CaSR changed a cysteine at amino acid 129 to a serine (Cys129Ser).[58,81] As this cysteine participates in dimerization of the CaSR, this result suggests that this cysteine constrains the receptor in its inactive state.

Clinical and genetic features of Bartter's syndrome subtype V (OMIM 601199.0035)

The Bartter-Gitelman spectrum of disorders is a heterogeneous syndrome of abnormal renal tubular transport characterized by defects in sodium and chloride re-absorption in the thick ascending limb of the loop of Henle (mimicking a furosemide-like effect). One key transporter involved in this spectrum of disorders is the apical Na-2Cl-K transporter that transports sodium and potassium down an electrochemical gradient. The efficiency of this transporter and the maintenance of a transepithelial voltage gradient is enhanced by potassium recycling across the apical renal outer medullary potassium channel (ROMK) that also drives the paracellular absorption of calcium and magnesium.

The overall feature of this class of disorders includes: renal salt wasting; hypokalaemic metabolic alkalosis; elevated renin and aldosterone levels; and normal to low blood pressure. In a small proportion of individuals with Bartter's syndrome subtype V, additional features of hypercalciuria, hypocalcaemia and hypomagnesaemia may be present and represent gain-of-

function mutations in the CaSR that further implicate the CaSR not only in calcium and magnesium homeostasis but also in salt and water homeostasis.[82] The CaSR-related cases of Bartter's syndrome identified to date have been inherited in an autosomal-dominant manner, unlike other subtypes that are inherited as autosomal-recessive traits.

In a 2002 case report, investigators found activating mutations of the *CaSR* gene in three patients involving L125P, C131W and A843E, which inhibited the activity of the ROMK channel that is mutated in Bartter's syndrome subtype II.[83,84] This observation provides the missing link that explains why some activating mutations of CaSR can cause the Bartter's syndrome phenotype. Another recent case report of this syndrome in monozygotic twins involving the K29E mutation in the ECD of the CaSR described mild hypokalaemia, minimal aldosterone and renin production, absent alkalosis but notable hypocalcaemia.[85] The K29E mutation has been reported to be a significant activating mutation of the CaSR,[86] and buttresses previous observations that the phenotype of Bartters syndrome is variable and not directly related to the in-vitro potency of the known genetic changes associated with this syndrome.[87]

The clinical presentation of Bartter's syndrome can occur in the perinatal period with polyhydramnios and premature delivery, or in the first few years of life with polyuria, polydypsia, isosthenuria or hyposthenuria, failure to thrive and frequent episodes of dehydration. The mainstay of treatment at the present time is non-specific and includes fluid and electrolyte replacement. Chronic hypokalaemia worsens the condition and can be ameliorated with amiloride or aldosterone antagonists. Magnesium repletion is frequently required.

POLYMORPHISMS OF THE CASR

In addition to clinically relevant activating and inactivating mutations, single nucleotide polymorphisms (SNPs) have been identified in the general population or in families with FHH and ADH in which the base pair change is present in affected and unaffected persons and does not segregate with known diseases of divalent ion metabolism. Six SNPs have been found in the *CaSR* gene; one in intron 5 just before exon 6 (IVS 5-88 t/c) and the remaining five in exon 7 in the coding region [one in the sixth TM (A826T), one in the seventh TM (C851S) and three in the ICD (A986S, R990G and Q1011E)]. The polymorphism in intron 5, IVS 5-88 t/c, is very common,[88] but no correlation has been found between this mutation and the incidence of parathyroid adenoma or diabetes.[89] The A826T mutation has been seen in 16% of 50 normal subjects' samples.[90] The C851S mutation was found in an ADH family in both affected and unaffected members,[79] but the investigators also found another mutation in this family (A116T) which segregates with the disease, and concluded that C851S was a rare polymorphism. The frequency of three common polymorphisms in the cytoplasmic tail varies in different populations. In one investigation of 377 unrelated DNA samples in a normal Caucasian (Italian) population, the relative frequencies for CaSR SNPs 986S, 990G and 1011E minor alleles were 24%, 4% and 3%, respectively.[91]

Interestingly, a study analysing serum calcium levels in samples from a normal population found that the homozygous polymorphism A986S was associated with higher serum calcium levels compared with the heterozygous form, while the heterozygous A986S had the lowest calcium levels, suggesting a spectrum of severity in phenotypic features and variable penetrance associated with various mutations.[91,92] Further analysis of tri-locus haplotypes for the A986, R990 and Q1011 alleles in the same study[91] showed that the highest ionized blood calcium levels were in subjects with the SRQ/ARE genotype compared with the ARQ/ARQ (wild-type) genotype, and suggests that tri-locus haplotyping may be more informative in studies of association between variation in CaSR and disease. At the present time, there is little evidence that the SNPs are disease causing, but there is the possibility that they may be

associated with increased risk for disease. For example, on the basis of a CaSR polymorphism haplotype study in stone-forming patients, it is suggested that the 990G variant could influence renal CaSR activation and calcium excretion.[93]

CASR-BASED THERAPEUTICS

Specific gene-based therapies for disorders of mineral metabolism as a result of mutations of the CaSR are not yet available. Similarly, there are currently no pharmacological agents approved by the Food and Drug Administration (FDA) for treatment of these disorders. However, clinically symptomatic disease as a result of these mutations could potentially be addressed with the use of modulators of the CaSR. The development of allosteric activators ('calcimimetics')[41] and antagonists ('calcilytics')[45] of the CaSR has made possible CaSR-based therapy of disorders of Ca^{2+}_o homeostasis (Table 1). One foreseeable problem with the use of modulators of the CaSR is the heterogeneity of response to treatment as a result of uniqueness of mutations and variability of binding of drug-ligand with mutant receptors.

One agent that could potentially be used in the management of disorders involving inactivating mutations of the CaSR (i.e. FHH and NSHPT) is AMG073, known as cinacalcet hydrochloride. It has recently been approved by the FDA for use in treating secondary hyperparathyroidism in patients receiving dialysis therapy for end-stage kidney disease,[94] as well as in parathyroid cancer. The drug also shows efficacy in mild primary hyperparathyroidism, as described below, but has not yet received FDA approval for this indication.

Calcium receptor antagonists, so-called calcilytics, have also been developed, and their clinical utility is being explored. Again, the FDA has not approved the use of these agents in the treatment of any disorders involving mutations of the CaSR. In the presence of a calcilytic, a higher than usual calcium concentration is needed to suppress PTH levels to a given extent. As a result, the calcium receptor reads normocalcaemia as hypocalcaemia and secretes a pulse of PTH. Similar adjustment in the sensing function of mutant 'activated CaSR' in the kidney or other CaSR-expressing tissues can be hypothesized to occur with use of calcylitics, as has been shown with wild-type CaSR in the parathyroid.[45,95]

SUMMARY AND FUTURE ISSUES

The CaSR is a membrane-bound 7TM receptor expressed in several tissues such as the parathyroid gland, kidney, gut and bone, and regulates Ca^{2+}_o homeostasis by acting as the body's 'calciostat'.

Patients who have loss-of-function mutations in the *CaSR* gene exhibit a form of hypercalcaemia that is accompanied by absolute calciuria or hypocalciuria. In the heterozygous form, it produces a benign hypercalcaemic condition, FHH. In the homozygous form (NSHPT), the hypercalcaemia may be lethal if it is not treated surgically.

Gain-of-function mutations produce a generally benign state of hypocalcaemia with relative or absolute hypercalciuria, as seen in ADH or Bartter's subtype V syndromes.

Polymorphisms of the CaSR exist in the general population but, by definition, are not associated with clinically relevant disease.

Disease-causing mutations of the CaSR can be associated with a spectrum of clinical phenotypes within and between kindreds.

At the present time, treatment for disorders of hyper- and hypocalcaemia due to mutations of the CaSR involve generally supportive measures.

Mutant CaSR is a target for therapeutic manipulation using gene-based therapy, which is currently unavailable. There is potential for use of pharmaceutical agents (calcimimetics and calcylitics). However, this approach is yet to be approved by drug-regulating bodies. One foreseeable problem with the use of modulators of the CaSR is the heterogeneity of response to treatment as a result of uniqueness of mutations and variability of binding of drug-ligand with mutant receptors.

It will be of interest in future studies to collect detailed clinical information in large kindreds to study the possible implications of the CaSR's altered sensitivity to calcium in these patients, and the resulting alterations in the levels of serum calcium. This could be even more relevant than previously considered, since the CaSR is expressed in numerous organs, such as the breast, brain, intestine and cardiovascular system, which are not thought to be involved in systemic calcium homeostasis.

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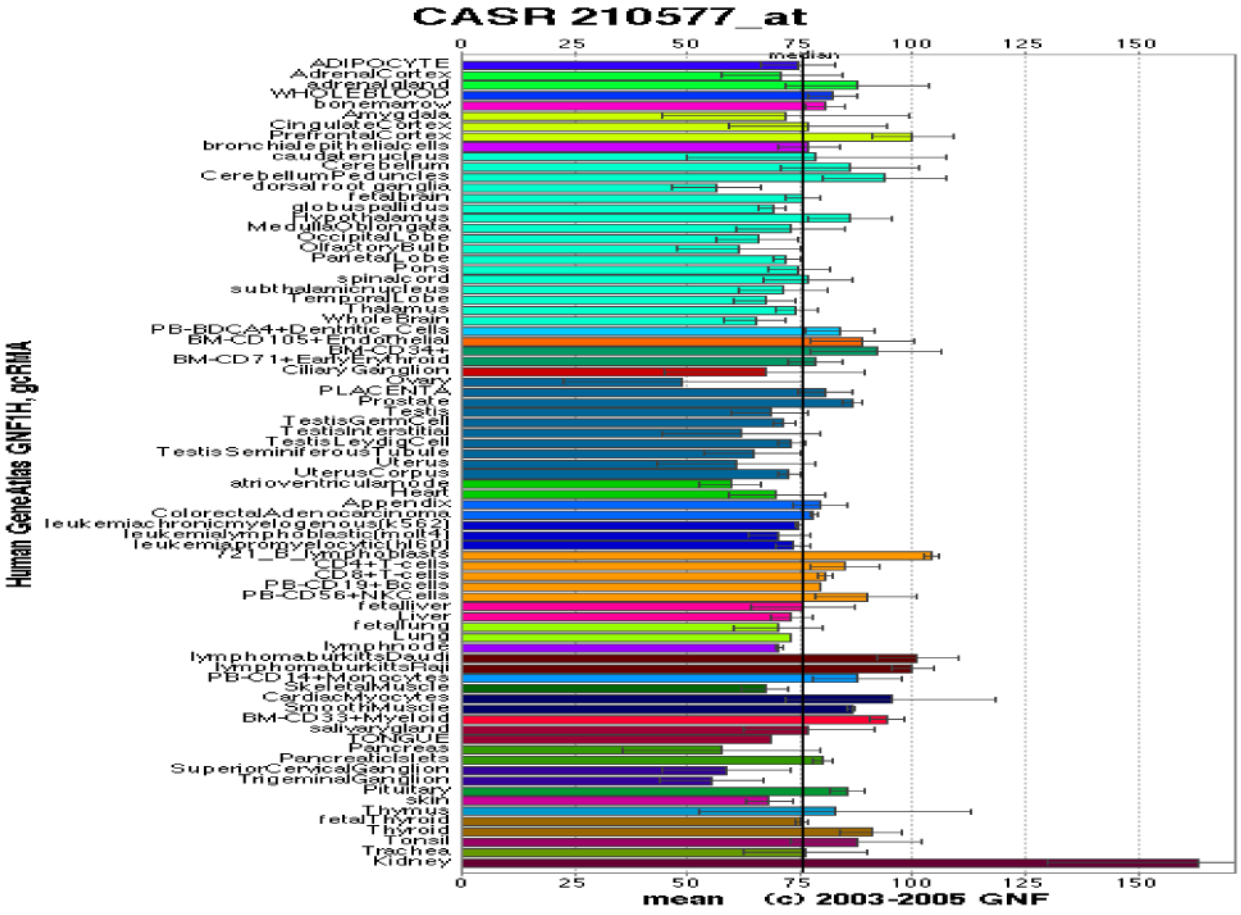

Figure 1.

Tissue distribution and density of expression of the calcium-sensing receptor. Adopted with permission from the Novartis Research Foundation Gene Atlas Database.[7]

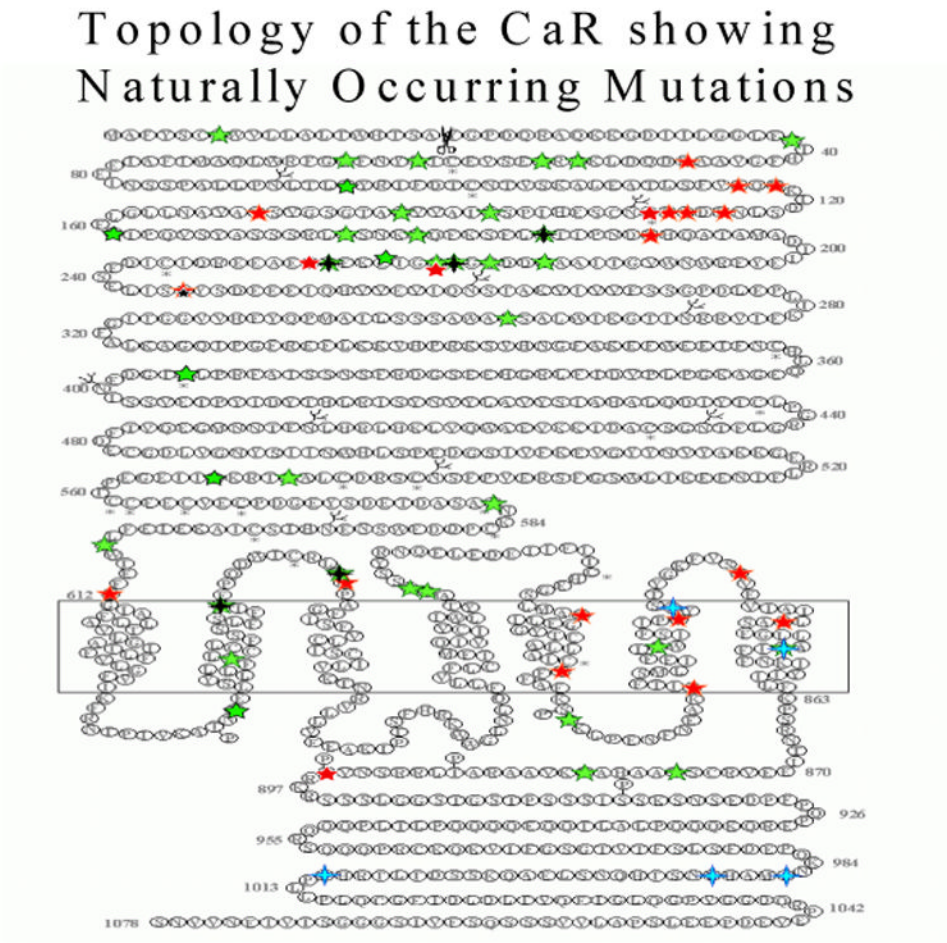

Figure 2.
Topology of the calcium-sensing receptor showing some of the naturally occurring mutations.

Table 1

Key roles of the calcium-sensing receptor in the parathyroid gland and kidney.

•	Parathyroid gland (1) Inhibit PTH secretion (2) Inhibit PTH gene expression (3) Inhibit parathyroid cellular proliferation
•	Kidney (1) Proximal tubule – blunt PTH-induced phosphaturia (2) MTAL – inhibit NaCl re-absorption (3) CTAL – inhibit re-absorption of Ca2+ and Mg2+ (4) IMCD – inhibit vasopressin-elicited water re-absorption

PTH, parathyroid hormone; MTAL, medullary thick ascending limb; CTAL, cortical thick ascending limb; IMCD, inner medullary collecting duct.

Table 2

Use of calcium-sensing receptor (CaSR)-based therapeutics.

- Calcimimetics
  (1) Approved by FDA
    (a) Secondary hyperparathyroidism in dialysis patients
  (2) Not yet approved
    (a) Primary hyperparathyroidism
    (b) Possibly FHH/NSHPT or inactivating CaSR antibodies
- Calcilytics
  (1) Not yet FDA approved
    (a) Osteoporosis
    (b) Possibly activating CaSR mutations or antibodies

FDA, Food and Drugs Administration; FHH, familial hypocalciurichypercalcaemia; NSHPT, neonatal severe primary hyperparathyroidism.