review article

The Calcium-Sensing Receptor and Related Diseases

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ABSTRACT

The calcium-sensing receptor (CASR) adjusts the extracellular calcium set point regulating PTH secretion and renal calcium excretion. The receptor is expressed in several tissues and is also involved in other cellular functions such as proliferation, differentiation and other hormonal secretion. High extracellular calcium levels activate the receptor resulting in modulation of several signaling pathways depending on the target tissues. Mutations in the *CASR* gene can result in gain or loss of receptor function. Gain of function mutations are associated to Autossomal dominant hypocalcemia and Bartter syndrome type V, while loss of function mutations are associated to Familial hypocalciuric hypercalcemia and Neonatal severe hyperparathyroidism. More than one hundred mutations were described in this gene. In addition to calcium, the receptor also interacts with several ions and polyamines. The CASR is a potential therapeutic target to treatment of diseases including hyperparathyroidism and osteoporosis, since its interaction with pharmacological compounds results in modulation of PTH secretion. **(Arq Bras Endocrinol Metab 2006;50/4:628-639)**

Keywords: Familial hypocalciuric hypercalcemia; Autosomal dominant hypocalcemia; Mutations; CASR; Bartter syndrome type V; Neonatal severe hyperparathyroidism

RESUMO

O Receptor Sensor de Cálcio e Doenças Associadas.
O receptor sensor de cálcio (CASR) ajusta o *set point* do cálcio extracelular através da regulação da secreção de PTH e da excreção renal de cálcio. O receptor é expresso em diversos tecidos e também está envolvido em outras funções celulares como proliferação, diferenciação e secreção de outros hormônios. Concentrações altas de cálcio extracelular ativam o receptor resultando em modulação de inúmeras vias de sinais intracelulares dependendo do tecido-alvo. Mutações no gene do CASR podem resultar em ganho ou perda de função do receptor. Mutações com ganho de função são associadas à Hipocalcemia autossômica dominante e à Síndrome de Bartter tipo V, enquanto que mutações com perda de função são associadas à Hipercalcemia hipocalciúrica familiar e ao Hiperparatireoidismo neonatal grave. Mais de cem mutações foram descritas neste gene. Além do cálcio, o receptor também interage com inúmeros íons e poliaminas. CASR é um alvo terapêutico potencial para tratamento de doenças incluindo hiperparatireoidismo e osteoporose, pois a sua interação com compostos farmacológicos resulta em modulação da secreção de PTH. **(Arq Bras Endocrinol Metab 2006;50/4:628-639)**

Descritores: Hipercalcemia hipocalciúrica familiar; Hipocalcemia autossômica dominante; Mutações; CASR; Síndrome de Bartter tipo V; Hiperparatireoidismo neonatal grave

ELECTROPHYSIOLOGICAL STUDIES SHOW that parathyroid cells possess a cell surface [Ca^{2+}_o] sensing mechanism that results in changes in phosphoinositide turnover and cytosolic calcium to regulate PTH secretion (1). Extracellular calcium regulates itself by serving as a first messenger and interacting with its receptor, the calcium-sensing receptor (CASR) on target tissues. The receptor was cloned in 1993 from bovine parathyroid (BoPCAR1) by expression cloning in *Xenopus laevis* oocytes and is a member of the G protein-coupled receptor super family (2). High calcium levels activate the CASR in the parathyroid cell surface to inhibit PTH secretion, and in the kidney to increase calcium excretion (3).

STRUCTURE OF THE CALCIUM-SENSING RECEPTOR

The human *CASR* gene is located on chromosome 3q13.3-21 (4,5) and spans over 50 kb of genomic DNA. It has a coding region of 3234 bp, which is contained within 6 exons (6). The human CASR is ~120 kDa protein, consisting of 1078 amino acid, with 612 amino acids in the extracellular domain (ECD), 250 amino acids of which comprise seven transmembrane spanning domains (TM), intracellular (ICL) and extracellular loops (ECL), and 216 amino acids of a long C-terminus cytoplasmic tail (ICD) (6). The CASR belongs to the metabotropic glutamate receptor subfamily, which comprises the metabotropic glutamate receptors (mGluR) (7), the $GABA_B$ receptor (8), the Vomero-nasal (pheromone) receptors (9), the taste receptors (10), the GPRC6A receptor (11) and five orphan receptors (12,13).

Studies, either with *CASR* cDNA transiently transfected in HEK293 cells (14) or expressed endogenously in rat inner medullary collecting duct endosomes (15), show that the CASR form homodimers via intermolecular disulfide linkages within the ECD. Dimers are the most abundant species present on the cell surface and intermolecular interactions within the dimeric CASR are important for receptor function (16,17). The ECD of the CASR contains nine potential N-linked glycosylation sites (6). The native CASR, as well as recombinant receptors transfected in HEK293 cells, present as three forms: 1) a 120 kDa band, which represents the non glycosylated species; 2) a 140 kDa band, which represents the immature glycosylated receptor; and 3) a 160 kDa band, which is the mature fully glycosylated receptor (18). Although the immature glycosylated receptor can reach the plasma membrane to a low extent, only the fully glycosylated receptor is functional (19,20).

Calcium binding sites

Due to the lack of a high-affinity ligand binding assay for the CASR, all the positions where calcium binds are unknown. Also, it is not known how many calcium ions bind to each receptor since there is the possibility of different affinity of each ligand binding domain for the ligand, cooperativity between the ligand binding sites and dimerization of the receptor (21). The pharmacology of the CASR is unusual for a receptor, as it only responds to the ligand in the millimolar ion concentration range, suggesting low affinity of the receptor for [Ca^{2+}_o] (22). However, this affinity range of the receptor is of physiological relevance as the free [Ca^{2+}] range is 0.75 to 2.0 mM for extracellular fluids (22). The ECD of the CASR has homologous regions that align with the mGluRs and the related bacterial periplasmic amino acid binding protein, suggesting that the CASR might have evolved from an ancient family of cell-surface proteins binding essential extracellular solutes, and suggesting the existence of additional ion-sensing receptors (23). The Venus flytrap model is the proposed model of ligand receptor interaction for the metabotropic glutamate receptor family (24). In this model two ligand-bound forms have been observed: an open conformation with the ligand initially bound to one ligand pocket in the large ECD with low affinity, and a closed form in which the ligand binds to a second domain stabilizing a high-affinity closed conformation, enclosed within the cleft. The liganded N-terminal segment interacts with the membrane-associated domain to generate a signal (24). Alignment of the extracellular domain of the CASR with the metabotropic glutamate receptor and the related bacterial amino-acid binding protein suggested that Ser 147 and Ser 170 correspond to residues in the binding pockets in the CASR (25). Further mutation analysis associated to molecular modeling studies indicated that calcium interact with polar residues in the binding pockets in the ECD of the receptor, with residues Ser 170, Asp 190, Gln193, Ser 296 and Glu 297 directly involved in Ca^{2+} coordination and residues Tyr 218 and Phe 270 and Ser 147 contributing to complete the coordination (26).

Calcium-sensing receptor agonists

Although calcium is the endogenous ligand for the CASR, it also shows affinity for a variety of di-, tri-, and polyvalent cations *in vitro* such as Mg^{2+}, Ba^{2+}, Sr^{2+}, Gd^{3+}, La^{3+}, neomycin, spermine, and protamine (6). The rank order of potency of some agonists is: Gd^{3+} > neomycin > Ca^{2+} > Mg^{2+}, with a half-maximal response (EC_{50}) of 20 μM, 70 μM, 3 mM and 10 mM, respectively (2). The

physiological relevance of the interaction of CASR with ions other than Ca^{2+} is unknown. In addition to cations, studies suggest that the CASR also senses sodium and ionic strength in parathyroid cells (27,28).

CALCIUM-SENSING RECEPTOR SIGNALING

When BoPCaR1 is expressed in *Xenopus laevis* oocytes, agonists elicit an increase in inositol 1,4,5-trisphosphate, which is completely blocked by treatment with pertussis toxin, indicating that the response is mediated through Gαi or $G\alpha_o$ (2). However, in bovine parathyroid cells, high levels of $[Ca^{2+}_o]$ activate phospholipase C (PLC) in a pertussis toxin-insensitive manner, suggesting that the CASR is coupled to PLC through a member of the Gq family (29). Interaction with Gαq is followed by activation of phospholipase Cβ, breakdown of phosphatidylinositol 4,5-bisphosphate with formation of 1,2-sn-diacylglycerol and of inositol 1,4,5-trisphosphate (IP3). The accumulation of IP3 leads to the release of intracellular pools of calcium contributing to intracellular signaling and causing inhibition of PTH secretion through mechanisms that remain to be fully defined (30). The high $[Ca^{2+}_o]$ also induces a sustained rise in $[Ca^{2+}_i]$ in parathyroid and in CASR transfected HEK293 cells associated with activation of a Ca^{2+}-permeable, nonselective cation channel (31). Activation of the receptor mediates different signal transduction pathways, depending on the cell line. In Chinese hamster ovary cells elicit phosphatidylinositol and arachidonic acid responses (32). In a mouse pituitary cell line (AtT-20) agonist-elicited increase in inositol phosphate is pertussis toxin-sensitive (33). In Madin-Darby canine kidney cell line, the CASR shows interactions of the receptor with Gαi-2, Gαi-3, and Gαq/11 (34). In the human astrocytoma cell line U87 (35), rat oligodendrocytes (36), rat microglia primary cultures (37), as well as in human lens-epithelial cells (38), the CASR activates an outwards K^+ channel. In rat fibroblasts, CASR was shown to mediate cell proliferation through an increase in c-SRC and ERK1 tyrosine kinases activity (39). In the human colonic cell line Caco-2, low Ca^{2+} (via interaction with CASR) induces proliferation and c-myc proto-oncogene expression via PKC activation (40).

ROLE OF THE CASR IN DIFFERENT TISSUES

Regulation of parathyroid function

The highest cell surface expression levels of CASR are found in parathyroid cells. CASR plays a crucial role in regulating PTH secretion and the parathyroid cells recognize remarkably small perturbations in the $[Ca^{2+}_o]$, and respond by altering the secretion of PTH (22). $[Ca^{2+}_o]$ has an inverse steep sigmoidal relationship with PTH secretion, and most of the sensing of $[Ca^{2+}_o]$ in parathyroid cells occurs over changes in free $[Ca^{2+}]$ of approximately 0.25 mM (22). The set point of normal human parathyroid, defined as the calcium concentration at which PTH secretion is half-maximal, is ~1 mM, and it plays an important role in determining the level at which $[Ca^{2+}_o]$ is set by the homeostatic system. Inactivating mutations in the CASR result in a mild increase in the set point for $[Ca^{2+}_o]$. In addition, $[Ca^{2+}_o]$ exerts several other actions on parathyroid function including modulation of the intracellular degradation of PTH, cellular respiration and membrane voltage, but the role of the CASR in mediating these effects is not known (31). Bovine parathyroid cells maintained in culture for more than 24 hours reduce dramatically their responsiveness to $[Ca^{2+}_o]$ (2). This is associated with a significant reduction in mRNA and protein levels of CASR (41).

Regulation of calcium excretion in kidney

Kidney is the major route for mineral ion excretion from the body and plays a key role in calcium homeostasis. In addition to PTH, the CASR plays an important role in regulating renal divalent mineral transport processes by both direct (by regulating calcium and water handling) and indirect (by modulating PTH secretion) mechanisms (42). $[Ca^{2+}_o]$ modulates renal tubular divalent mineral and water transport processes by interacting with the CASR (42).

The CASR has been localized within several segments of the rat tubule, but it is expressed at highest levels in the cortical thick ascending limb (CTAL) (43). It is found mostly on the basolateral surface of tubular cells, but also to a lesser extent on the apical surface (31). Elevated peritubular $[Ca^{2+}_o]$ and $[Mg^{2+}_o]$ reduces the tubular reabsorption in isolated microperfused segments of CTAL *in vitro* (44). The reabsorption of Ca^{2+} and Mg^{2+} in CTAL occurs mainly through a paracellular pathway driven by a lumen-positive, transepithelial potential generated by the transport of Na^+, K^+, and Cl^- by the apical Na-K-2Cl co-transporter combined with recycling of K^+ into the lumen via an apical K^+ channel (31). While PTH acts through its receptor in the kidney, stimulates cAMP accumulation, enhances the co-transport activity and results in an increase of Ca^{2+} and Mg^{2+} transport, $[Ca^{2+}_o]$ inhibits the activity of the apical K^+ channel resulting in a decrease in

co-transporter activity and a reduction of Ca^{2+} and Mg^{2+} transport (31). High $[Ca^{2+}_o]$ in the mouse CTAL decreases hormone-dependent cAMP accumulation as a result of a direct inhibition of adenylyl cyclase (AC) activity (45). An increase in Arginine vasopressin (AVP)-elicited osmotic water permeability in collecting ducts stimulates water reabsorption selectively via aquaporin-2 (AQP-2) water channels (46). CASR and AQP-2 were also found to co-express in rat kidney inner medullary collecting ducts (IMCD) suggesting a direct effect of CASR in inhibition of AVP-elicited osmotic water permeability and the consequent increase in diuresis (46). CASR and Ca^{2+}-inhibitable AC were found to co-express and co-localize in the rat CTAL cells (47), and cAMP synthesis is inhibited by agents coupled to PLC or to Gαi protein-mediated process suggesting that the CASR contributes to the effect observed for high $[Ca^{2+}_o]$ (47). Additional evidence of the role of CASR in regulating Ca^{2+} and Mg^{2+} transport in CTAL is found in subjects with mutations in the *CASR* gene. In subjects with FHH due to an inactivating mutation in the *CASR* there is a PTH-independent increase in tubular Ca^{2+} reabsorption (48), while in ADH subjects there is increased urinary calcium excretion (31).

Role of the calcium-sensing receptor in other tissues

The CASR is widely distributed and is also found in tissues that are not directly involved in calcium homeostasis. In these tissues it appears that high $[Ca^{2+}_o]$, via interaction with CASR, regulates a series of cellular functions such as increased cell proliferation in fibroblasts (39), induction of cell differentiation in keratinocytes (49) and human colon epithelial cells (50), prevention of apoptosis in AT-3 prostate carcinoma cells (51), and cataract formation in lens epithelial cell (38). CASR was detected in a murine bone marrow-derived stromal cell line (ST2) (52), in osteoblast-like cell lines (53) and in rabbit mature osteoclasts (54), however its role in bone is still debatable (55,56). In the mouse pituitary cell line, AtT-20 cells, it was shown that the CASR was implicated in adrenocorticotropic hormone (ACTH) (57) and α-MSH release (58). The CASR was also demonstrated in human insulinoma primary cultures, causing released insulin upon $[Ca^{2+}_o]$ stimulation (59), in hepatocytes stimulating bile flow (60) and in antral gastric cells stimulating gastrin secretion (61).

DISEASES ASSOCIATED WITH MUTATIONS IN THE CASR

Disorders due to loss of the calcium-sensing receptor function

Two autosomal disorders, Familial Hypocalciuric Hypercalcemia (FHH) and Neonatal Severe Primary Hyperparathyroidism (NSHPT), have been associated with loss of CASR function due to inactivating mutations.

Familial hypocalciuric hypercalcemia

FHH is characterized by moderate elevations of serum calcium concentration (hypercalcemia), lower urinary calcium excretion (hypocalciuria) and inappropriately normal parathyroid hormone (PTH) levels (62,63). This is not a life-threatening condition and most of the usual sequelae of hypercalcemia such as altered mental status, kidney stones, decreased urinary concentrating ability and hypertension are absent (64). Patients are usually asymptomatic or have nonspecific symptoms such as fatigue, weakness, painful joints and headache, with the diagnosis only suspected after a routine biochemical screening showing high blood calcium levels (63). Interestingly, some subjects with FHH present with an incomplete phenotype, lacking hypocalciuria. In some families a more severe phenotype suggestive of familiar isolated hyperparathyroidism is present (65,66). FHH is inherited as an autosomal dominant disorder, and all affected individuals with mutations in the *CASR* gene are heterozygous for the mutation (67). The dominant pattern of inheritance of this disease has been attributed to haploinsufficiency of the *CASR* gene, where protein receptor produced by a single normal allele cannot support normal function, although it may suffice for survival (68).

The gene responsible for FHH was linked to chromosome 3q 21-24 in four families (69) and later fluorescence *in situ* hybridization analysis identified the position of the gene as 3q 13.3-21 (4). Cloning of the *CASR* was followed by reports of inactivating mutations in this gene in FHH families (70,71). FHH is a heterogeneous disease, and the disease locus segregates with chromosome 3 in most of the families (FHH type 1); however, mutations in other genes may be responsible for similar phenotypes as the disease also segregates to chromosome 19p13.3 (72) in one family (FHH type 2) and 19q13 (73) in another family (FHH type 3) (74). In this last case, besides hypercalcemia and hypocalciuria, affected individuals present increase in PTH serum levels, hypophosphatemia and osteomalacia. In view of the lack of complications,

medical treatment for lowering the calcium level is not indicated (75). Surgical exploration of the parathyroid glands is also not indicated, as parathyroidectomy does not cure the disorder (63).

Neonatal Several Hyperparathyroidism

NSHPT (76,77) represents the most severe expression of familial hypocalciuric hypercalcemia (68). In most patients in which mutations were found in the *CASR*, the two gene copies are mutated, with both parents having passed on a mutated copy and presenting with FHH. There are three reports of mutations being found *de novo* in individuals with NSHPT with only one copy mutated and no mutation found in the parents (78,79). Neonatal severe hyperparathyroidism causes a marked elevation in serum calcium and PTH levels. It appears very early, in the first days of life, and the baby presents with hypotonia, poor feeding, failure to thrive and respiratory distress associated with rib cage deformities (80). PTH concentrations are very high, associated with calcium levels that are life-threatening (80). In severe cases, surgical intervention is essential, with total parathyroidectomy still being the currently accepted method of treatment. However, there are reports of cases where symptoms are not life threatening and could be controlled using medical therapy to maintain calcium at levels compatible with normal life (81,82).

Disorders due to gain of calcium-sensing receptor function

An autosomal dominant hypocalcemia (ADH) and Bartter syndrome type V have been associated with gain of CASR function due to activating mutations in the receptor.

Autosomal Dominant Hypocalcemia

ADH presents with a wide clinical spectrum, from severe hypocalcemia in the neonatal period to an incidental finding in adulthood (83). Associated problems include seizures, mental deficiency, orodental problems, basal ganglia calcification, kidney stones and renal failure (84). Individuals present with hypocalcemia, hyperphosphatemia, low serum PTH levels and hypercalciuria (84). Autosomal dominant hypocalcemia was initially classified as familial isolated hypoparathyroidism, a heterogeneous group of disorders characterized by PTH deficiency, hypocalcemia and hyperphosphatemia. Within this group, different modes of inheritance were identified with transmission patterns consistent with autosomal dominant, autosomal recessive, and X-linked forms. Finegold et al. linked one form of autosomal dominant hypoparathyroidism to chromosome 3q13 (85) and Pollak et al. (86) described the first activating mutation in the *CASR* in a family with ADH, and the terminology was recommended to be changed to autosomal dominant hypocalcemia, as a direct contrast to the hypercalcemia in FHH (87). In most individuals where mutations have been found, familial inheritance is clear, with one parent being affected with the same mutation (67). However, *de novo* mutations found in individuals where no mutation was found in the parents have also been described (67). A careful treatment for this condition is required, as attempts to normalize blood calcium levels with regular doses of vitamin D tend to exacerbate urinary calcium levels and increase the risk of kidney stone and renal impairment (88). Treatment should be limited to symptomatic patients. Hydrochlorothiazide has been used to control hypercalciuria in these patients (88). Recombinant human PTH to improve hypocalcemia symptoms has been described, however longer follow-up studies are required (89).

Bartter Syndrome type V

Bartter syndrome is a heterogeneous rare disease due to deficiency in sodium and chloride absorption. Biochemical profile is renal salt wasting, hypokalemic metabolic alkalosis, elevated renin and aldosterone levels with low blood pressure. In some individuals hypercalciuria is also present. Gain of function mutations in the CASR has been described in some patients with Batter syndrome associated to hypocalcemia and hypercalciuria (90,91). Functional studies showed that these mutations (L125P, C131W and A843E) result in a more severe receptor activation when compared to other activating mutations described (90,91). Of interest, the mutation A843E is the only constitutive mutation described in the CASR, presenting a high basal activity in the absence of $[Ca^{2+}_o]$ (92). Clinical data in the literature may be biased towards the most severely affected individuals in both ADH and FHH/NSHPT and may not reflect the whole spectrum of the disease.

Calcium-sensing abnormalities in other disorders

Autoimmune hypoparathyroidism

Autoimmune hypoparathyroidism (AH) manifests biochemically by hypocalcemia and hyperphosphatemia caused by a deficiency of PTH. It represents

an integral part of type I autoimmune polyglandular syndrome, a rare disorder characterized by the presence of AH, Addison's disease, and mucocutaneous candidiasis and can be associated with female primary hypogonadism, keratopathy, alopecia, vitiligo, parietal cell atrophy, insulin-dependent diabetes mellitus, autoimmune hepatitis and hypothyroidism (93). In one study, an epitope within the ECD of the CASR was specifically recognized in 14 of 25 individuals (56%) with AH, suggesting that the CASR is a key antigen in directing the immune response against parathyroid tissue in this condition (94). The mechanism of the hypoparathyroidism is destruction of the parathyroid gland due to the inflammatory reaction and complement fixation.

Autoimmune hypocalciuric hypercalcemia

The ECD of the CASR is also antigen for antibodies that instead of inducing parathyroid cell destruction, interferes with the normal activation of the receptor resulting in increase PTH levels (95). Patients may manifest clinically with hypercalcemia, not suppressed PTH levels and hypocalciuria similar of FHH patients. However, it is not associated to mutation in the *CASR* gene (95). In addition to the hypercalcemia, patients may present other autoimmune disease such as thyroiditis, celiac sprue, psoriasis, hypophysitis, uveitis and rheumatoid arthritis (95).

Mutations in the calcium-sensing receptor

One hundred and twelve mutations (98 missense, 6 nonsense, 8 insertion and or deletion, and 1 splice mutation) have been described in the CASR mutation database (http://www.casrdb.mcgill.ca) related to FHH, NSHPT, ADH families or as *de novo* disease (figure 1) (96). In addition, 6 polymorphisms were found in samples from a normal population or in families with FHH and ADH in which this base pair change was present in affected and unaffected members and did not segregate with the disease. Fifteen mutations were found more than once in the *CASR* gene in apparently unrelated families. In several positions two different mutations were described in the same codon with the same receptor phenotype, with one exception. At position 297 an activation mutation (E297D) and an inactivating mutation (E297K) were described (26,70) confirming the crucial role of this position on receptor activation. Most of the mutations found in the ECD are located in the first third of the N-terminus suggesting the importance of this region in ligand binding. Activating mutations in the proximal 1/3 of the ECD may facilitate the ligand-binding interaction in the different binding sites, increasing the receptor affinity to the ligand, whereas inactivating mutations may have the opposite effect, disrupting the ligand binding pockets. This is supported by *in vitro* functional analyses of mutations in this location that show ligand-dependent changes in the affinity of the receptor to extracellular calcium (67).

Mutation in the TM domain may abrogate constraints, tilting the TM and locking the receptor in either an inactivating or activating conformation, as residues in the TM7 are critical for maintaining the receptor in an inactive conformation (97). From functional analyses of receptors with gain of function, only A843E showed the ability to activate the receptor in the absence of the ligand (92). The other activating mutations all showed a ligand-dependent shift of the dose-response curves to the left. This suggests that the mechanism of activation of the receptor in most of the TM domain mutations is to facilitate the TMD activation, with the exception of A843E that most likely locks the TMD in an active conformation. Inactivating mutations resulting in total loss of function of the receptor may also be associated to total loss of ability of the ligand bind and activate the receptor, even though the receptor is well expressed in the plasma membrane or due to misfolding and retention within the cytoplasm resulting in lack of receptor at the membrane (98).

The ICD seems to be important for receptor trafficking to the cell membrane and for the interaction with intracellular proteins. Large deletion of the c-terminal tail was associated to gain of function in an ADH family (99). *In vitro* functional studies showed gain of function and increase mutant receptor cell surface expression level. Mutagenesis in the ICD confirms its involvement in degradation and processing of the receptor (99). Residues 962-981 in the c-terminal tail are critical for its interaction with filamin A and this interaction prevents the receptor degradation and facilitates MAPK signaling (100). In contrast, interaction of the ICD with dorfin targets the receptor for degradation (101).

Activating mutations

Forty activating mutations in the *CASR* gene have been described. The majority is missense mutation, with 2 deletions described. Most ADH affected individuals are heterozygous for the activating mutation. In one family, homozygous mutation is described but it is not associated to a more severe phenotype (99). Clinical data from affected individuals with activating mutations are abundant and, despite the spectrum of severity of the phenotype for the same genotype, sim-

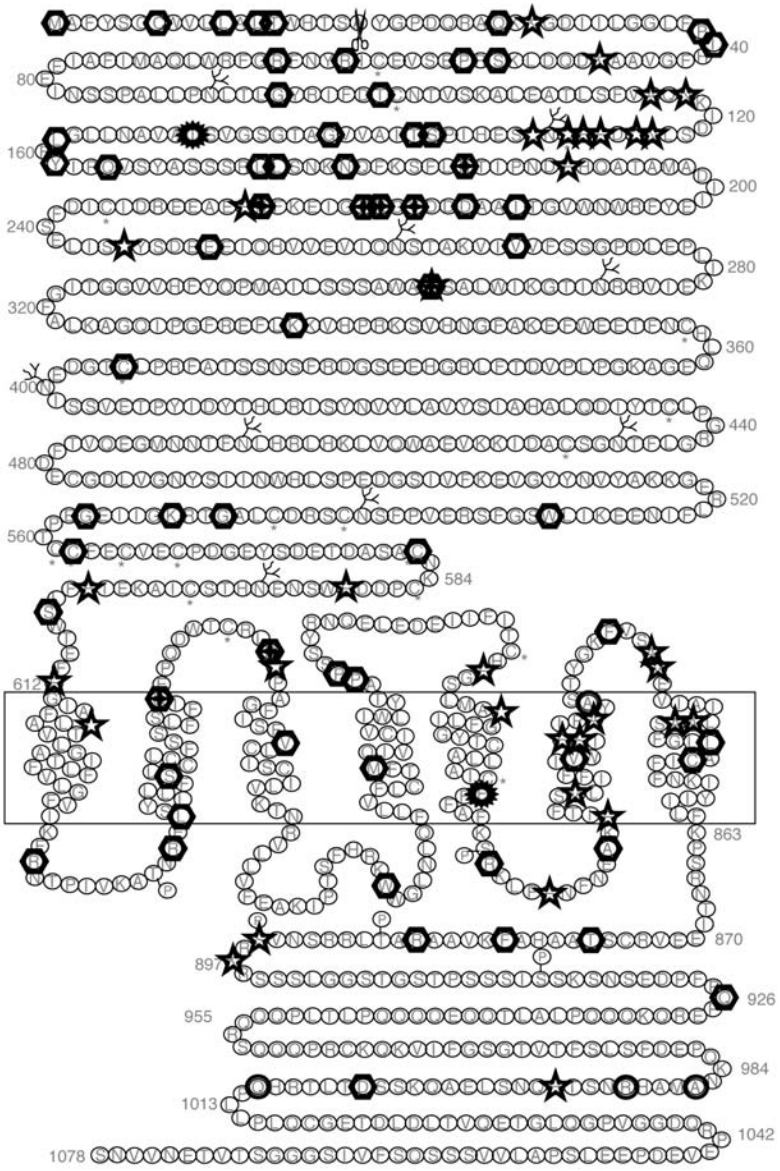

Figure 1. Topography and positions of mutations in the CASR. Symbols ✪ indicate positions with one inactivating mutation, ⊕ positions with two different inactivating mutations, ✯ positions with one activating mutation, ✡ positions with two different activating mutations and ⚬ indicate positions with polymorphisms. Adapted from D'Souza-Li in CASR mutation database, 1999. Reproduced with permission.

ilar symptoms are found in different families.

Inactivating mutations

Of the 72 inactivating mutations in the *CASR* gene, 59 are missense, 6 are nonsense, 6 are insertions and/or deletion including an Alu element insertion (102) and one splice mutation (103). The gene dosage effect is clear in most FHH cases, with one mutated gene copy resulting in FHH with mild hypercalcemia and two mutated copies resulting in NSHPT, a more severe phenotype that manifests very early in life with severe hypercalcemia, bone demineralization and failure to thrive (68). However, the three cases of *de novo* NSHTP reported in the literature were heterozygous for missense mutations located in the extracellular domain, with only one mutated allele and no mutation found in the parents (79,104). One individual with *de novo* NSHPT was heterozygous for a previously described mutation in a FHH family (79).

Polymorphisms

Six polymorphisms were 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 6th TM [A/T826], one in the 7th TM [C/S851], and three in the ICD [A/S986, R/G990 and Q/E1011]). The polymorphism in intron 5, IVS 5 -88 t/c, is very common (105) and, when analyzed in a large group of normal and affected individuals, no correlation was found between this mutation and the incidence of parathyroid adenoma or diabetes (106). The A/T 826 mutation was initially found in 4 parathyroid adenomas (107). Further analysis showed the same change in 16% of 50 normal subjects' samples (108). The C/S 851 was found in an ADH family in both affected and unaffected members (109). They also found another mutation in this family (A116T), which segregates with the disease, and concluded that C/S 851 was a rare polymorphism. The frequency of the 3 common polymorphisms in the cytoplasmic tail varies in different populations. In a large series in a Caucasian population the incidence for A/S986 was 24%, for R/G990 was 4% and for Q/E1011 was 3% within 377 unrelated DNA samples (110). In addition, a study analyzing serum calcium levels in samples from a normal population found that the homozygous polymorphism 986S was associated to higher serum calcium levels when compared to the heterozygous form, while the homozygous 986A had the lowest calcium levels (110,111).

INTERACTION OF THE CASR WITH PHARMACOLOGICAL COMPOUNDS

Calcimimetics

The CASR is also a target for pharmacological compounds that act synergistically with calcium as positive allosteric modulators of the receptor, such as NPS R-568 and cinalcalcet (112,113). However, these compounds require the presence of calcium and act by increasing the sensitivity of the receptor to $[Ca^{2+}_o]$ (114). Their interaction sites are in the TMD of the receptor with Glu 837 critical for their action (115). Clinical studies using a calcimimetic in secondary hyperparathyroidism showed significant reduction on PTH, and in the calcium x phosphate product levels in patients treated for 26 weeks (116). Cinacalcet was approved by the US FDA for the treatment of secondary hyperparathyroidism (113). Other potential uses for calcimimetics are co-adjuvant in the treatment of hyperparathyroidism and parathyroid carcinoma.

Calcilytic

Compounds that interact with the CASR as negative allosteric modulators have also been developed, such as NPS-2143 (117) and compound 1 (118). These compounds are potent CASR antagonists resulting in transient increase PTH secretion and bone formation (117). Calcilytic compounds are potential therapeutic agents for the treatment of osteoporosis, since they can reproduce the anabolic effect in bone of transitory increase in PTH.

CONCLUSION

The CASR plays an important role in regulating $[Ca^{+2}_o]$. The receptor is more versatile than ever expected, being involved in a variety of cellular function. It is also target to new pharmacological compounds that modifies its function with potential therapeutic applications.

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