Minireview

The role of calcium-sensing receptor signaling in regulating transepithelial calcium transport

Rebecca Siu Ga Tan[1,2], Christy Hui Lin Lee[1], Henrik Dimke[3,4] and R Todd Alexander[1,2,5]

[1]Department of Physiology, University of Alberta, Edmonton T6G 1C9, Canada; [2]Membrane Protein Disease Research Group, University of Alberta, Edmonton T6G 1C9, Canada; [3]Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense 5000, Denmark; [4]Department of Nephrology, Odense University Hospital, Odense 5000, Denmark; [5]Department of Pediatrics, University of Alberta, Edmonton T6G 1C9, Canada
Corresponding author: R Todd Alexander. Email: todd2@ualberta.ca

Impact statement

This minireview illustrates the role of calcium-sensing receptor signaling in transepithelial calcium transport across the kidney and intestine. Regulation of calcium transport contributes to the maintenance of calcium homeostasis and understanding the molecular mechanisms involved will provide insight for the treatment of diseases caused by perturbations in calcium balance.

Abstract

The calcium-sensing receptor (CaSR) plays a critical role in sensing extracellular calcium (Ca^{2+}) and signaling to maintain Ca^{2+} homeostasis. In the parathyroid, the CaSR regulates secretion of parathyroid hormone, which functions to increase extracellular Ca^{2+} levels. The CaSR is also located in other organs imperative to Ca^{2+} homeostasis including the kidney and intestine, where it modulates Ca^{2+} reabsorption and absorption, respectively. In this review, we describe CaSR expression and its function in transepithelial Ca^{2+} transport in the kidney and intestine. Activation of the CaSR leads to G protein dependent and independent signaling cascades. The known CaSR signal transduction pathways involved in modulating paracellular and transcellular epithelial Ca^{2+} transport are discussed. Mutations in the CaSR cause a range of diseases that manifest in altered serum Ca^{2+} levels. Gain-of-function mutations in the CaSR result in autosomal dominant hypocalcemia type 1, while loss-of-function mutations cause familial hypocalciuric hypercalcemia. Additionally, the putative serine protease, FAM111A, is discussed as a potential regulator of the CaSR because mutations in FAM111A cause Kenny Caffey syndrome type 2, gracile bone dysplasia, and osteocraniostenosis, diseases that are characterized by hypocalcemia, hypoparathyroidism, and bony abnormalities, i.e. share phenotypic features of autosomal dominant hypocalcemia. Recent work has helped to elucidate the effect of CaSR signaling cascades on downstream proteins involved in Ca^{2+} transport across renal and intestinal epithelia; however, much remains to be discovered.

Keywords: Calcium-sensing receptor, calcium homeostasis, calcium transport, kidney, intestine, calciotropic hormones, signaling cascades, FAM111A

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Introduction

The calcium-sensing receptor (CaSR) is a G-protein coupled receptor (GPCR) central to the maintenance of calcium (Ca^{2+}) homeostasis in vertebrates.[1,2] As the name implies, it senses the extracellular Ca^{2+} concentration and signals to maintain it within a narrow physiological range (Figure 1).[3] Central to this role, CaSR signaling occurs in the parathyroid glands,[4] kidneys,[5] intestine, [6] and bone.[7,8] The CaSR is highly expressed in the parathyroid glands,[9] where it regulates the synthesis and secretion of parathyroid hormone (PTH).[10] In response to low serum Ca^{2+} levels, PTH is secreted and acts to restore levels by (i) mobilizing Ca^{2+} from bones via stimulation of osteoclastic bone resorption,[11] (ii) promoting renal Ca^{2+} reabsorption,[12] and (iii) stimulating the synthesis of active vitamin D (1, 25-dihydroxyvitamin D), which in turn increases intestinal Ca^{2+} absorption.[13,14] Ca^{2+}-induced activation of CaSR signaling in the parathyroid gland acts to reduce circulating PTH levels by decreasing PTH gene expression,[15] PTH secretion,[16] and parathyroid cell proliferation.[17]

However, CaSR signaling in Ca^{2+} transporting epithelia directly regulates transport independently of PTH.

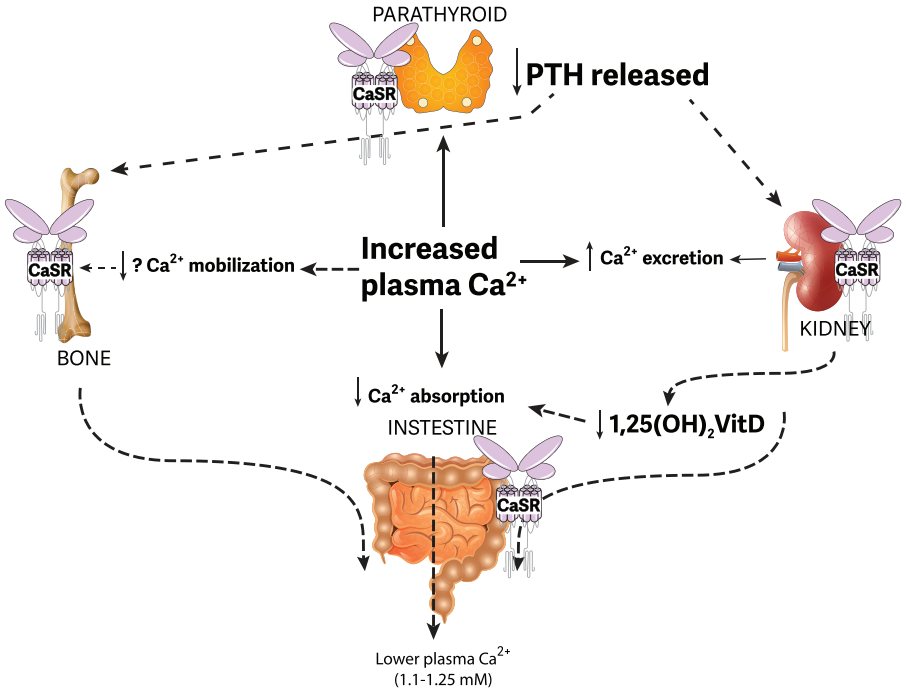

Figure 1. Schematic diagram of calcium (Ca^{2+}) homeostasis. Increased plasma Ca^{2+} activates the calcium-sensing receptor (CaSR; protein structure represented in diagram in purple as a homodimer) in the parathyroid decreasing parathyroid hormone (PTH) release. Decreased PTH release, in addition to CaSR activation in the bone and kidney, results in decreased Ca^{2+} release from the bone and increased Ca^{2+} excretion in the urine. Decreased PTH indirectly causes decreased Ca^{2+} absorption from the small intestine by reducing the kidney production of 1,25-dihydroxyvitamin D ($1,25(OH)_2$VitD). CaSR activation in the small intestine also directly decreases Ca^{2+} absorption. Overall, increased plasma Ca^{2+} activates the CaSR in multiple organs decreasing PTH, as well as directly affecting Ca^{2+} transport in the kidney, bone, and intestine to lower plasma Ca^{2+} to a normal physiological range (1.1–1.25 mmol/L). (A color version of this figure is available in the online journal.)

Studies on PTH null mice and thyroparathyroidectomized rats have revealed PTH-independent effects of CaSR activation on the extracellular Ca^{2+} concentration.[5,18] Activation of the renal CaSR increases urinary Ca^{2+} excretion by regulating the paracellular permeability to Ca^{2+} via modulation of claudin expression. Claudins are four-pass transmembrane proteins localized to the tight junction that regulate paracellular ion permeability.[19] In fact CaSR activation in the kidney increases claudin-14 (*CLDN14*) expression, which reduces the paracellular permeability of the epithelium to divalent cations (i.e. Ca^{2+} and Mg^{2+}) in the thick ascending limb (TAL).[20] In this segment, CLDN14 physically acts as a paracellular barrier to block the movement of divalent cations through the cation selective pore formed by claudin-16 (CLDN16) and claudin-19 (CLDN19).[20–22] Whether transcellular transport is affected by CaSR activation in the kidney is still disputable as there is debate on the localization of the CaSR in the nephron, outside the TAL. Activation of the intestinal CaSR reduces transcellular Ca^{2+} absorption,[6] while it remains to be determined whether paracellular Ca^{2+} absorption is directly affected by intestinal CaSR signaling.

The CaSR is also expressed in osteoclast and osteoblast cells of bone and participates in extracellular Ca^{2+} sensing within the bone microenvironment to modulate bone remodeling and extracellular Ca^{2+} levels.[7,8] High extracellular Ca^{2+} and CaSR agonists activate the CaSR in these bone cells, stimulating the proliferation and chemotaxis of bone-forming osteoblasts[7] while decreasing bone resorption by inducing apoptosis of osteoclasts.[8] Interestingly, osteoblasts have an additional G protein-coupled calcium sensor, GPCR6a, which also contributes to stimulating osteoblast-mediated bone formation.[23]

Given the central role of the CaSR in regulating blood Ca^{2+} levels, it is therefore not surprising that mutations in the *CASR* cause diseases that manifest as an inability to maintain Ca^{2+} homeostasis. Heterozygous inactivating mutations result in familial hypocalciuric hypercalcemia (FHH), a disease characterized by high serum Ca^{2+} and low urine Ca^{2+} levels with inappropriately normal or mildly elevated serum PTH.[24] Conversely, heterozygous activating mutations in the *CASR* cause autosomal dominant hypocalcemia (ADH) type 1, a disease characterized by low serum Ca^{2+}, high urinary Ca^{2+}, and inappropriately low serum PTH.[25] Herein we review the role of CaSR signaling, where it is known, in regulating transepithelial Ca^{2+} transport in the kidney and intestine. The localization of the CaSR in these organs and the effect of *CASR* mutations on Ca^{2+} homeostasis are also discussed. Additionally, we describe the potential role of FAM111A, a predicted serine protease, in CaSR modulation.

Localization of the CaSR in the kidney

Initial CaSR localization studies conducted on rat kidney found CaSR messenger RNA (mRNA) expression in most nephron segments including the glomerulus, proximal tubule (PT; convoluted and straight), TAL (cortical and medullary), distal convoluted tubule (DCT), and collecting ducts (CD; cortical and medullary).[26] Subsequently, CaSR protein expression was identified in rat kidney via

immunofluorescence microscopy, within the PT (convoluted and straight), TAL (cortical and medullary), DCT, and cortical CD.[27] For many years, however, it has been debated whether the CaSR was expressed in tubule segments other than the TAL.

Among the CaSR localization studies performed to date on rodent and human kidneys, there is consensus that CaSR transcript and protein is predominately expressed in the TAL with protein localization to the basolateral membrane. A study using human, rat, and mouse kidney only found CaSR transcript and protein in the TAL of the nephron.[5] Some, but not all investigations have also identified CaSR expression in the apical and basolateral membranes of the DCT and CD.[28–30] Others have failed to find expression in the glomerulus[30] and PT.[29,30] A recent study by Graca *et al*.[31] sought to determine the intrarenal distribution of the CaSR in mouse, rat, and human nephron segments using recent technological advances. They used a combination of methods including *in situ* hybridization, immunohistochemistry with eight different CaSR antibodies, and proximity ligation assay. mRNA was found in the TAL, DCT, and CD with the highest expression in the TAL. Protein was found all along the nephron including the glomeruli, PT (apical and cytoplasm; expression decreases from S1 to S3), TAL (basolateral and cytoplasm), connecting tubule, DCT (apical, basolateral, cytoplasm), and CD (apical, basolateral, cytoplasm).[27,31] The expression pattern was similar between mouse, rat, and humans, consistent with a high degree of conservation. The lowest CaSR expression was found in the glomeruli, then the PT, with the highest in the TAL. Due to discrepancies in studies over gene and protein expression of the CaSR along the nephron, other than the TAL, we have therefore focused on signaling in this segment. The predominant expression of the CaSR in the TAL suggests it plays an important functional role in this segment. Indeed, a function for the CaSR in the cortical TAL has been confirmed via *ex vivo* microperfusion studies employing peritubular addition of CaSR agonists, which elicited a Ca^{2+} signaling response resulting in increased cytosolic free Ca^{2+}.[5,32] Localization combined with functional studies is required to determine the presence and role of the CaSR in each nephron segment.

Localization of the CaSR in the intestine

There are fewer studies examining the localization of the CaSR in the intestine than in the kidney. The intestine, nevertheless, plays an important role in Ca^{2+} homeostasis as the site of Ca^{2+} absorption from the diet. Using reverse transcription-polymerase chain reaction (RT-PCR) and complementary northern blotting, CaSR transcripts have been identified in whole and mucosal samples of rat intestinal segments including the duodenum, jejunum, ileum, cecum, and colon.[33,34] Additionally, *in situ* hybridization on the duodenum found CaSR mRNA present in both crypt and villus cells, as well as the submucosa.[34] Immunohistochemistry of rat small intestinal segments identified CaSR protein localized predominately in the basolateral region of villi and crypt epithelial cells, with modest apical expression in villi.[34] Interestingly, CaSR mRNA and protein were also observed in the enteric nervous system of the small and large intestine.[34,35] Protein was found in the submucosa and serosa along the intestine.[34] In the rat colon, surface and crypt cells showed apical and basolateral expression of CaSR transcript and protein.[34,36] Additionally, the immunostaining pattern of the CaSR in colonic crypts was similar between human and rat.[36] CaSR transcript and protein has also been observed in several human colonic cell lines (Caco-2, HT-29 and T84).[33]

CaSR activation and signaling

CaSR ligands and activation: The CaSR is a class C GPCR composed of a large extracellular domain (ECD), seven transmembrane spanning domains, and an intracellular domain.[1,37–39] Homodimerization occurs by connection of the ECDs via a covalent disulfide linkage between cysteine residues in addition to non-covalent intermolecular interactions.[11,40,41] Crystal structures of the ECD of the CaSR show that it contains a large Venus flytrap module (VFTM) with two lobes (LB1 and LB2) that create a cleft for ligand binding.[37,42] The cysteine rich domain, which is present in most class C GPCRs, links the VFTM to the transmembrane spanning region.[37,43] Agonist-induced VFTM closure along with the presence of a cysteine-rich domain is required for CaSR activation.[37,43]

Extracellular Ca^{2+} is the primary endogenous agonist of the CaSR.[44] It binds within the VFTM.[44] Other agonists that bind the orthosteric site with varying affinity include divalent and trivalent cations, polyamines, and L-amino acids.[37,44,45] In addition to binding within the VFTM, the L-amino acids L-phenylalanine and L-tryptophan can also bind to allosteric sites and act as positive modulators by increasing the receptor's sensitivity to extracellular Ca^{2+}.[46,47] Mutations in residues that bind L-amino acids result in reduced CaSR-mediated response to extracellular Ca^{2+}, illustrating the importance of L-amino acid involvement in receptor activation.[46] Interestingly, L-amino acids only contribute to receptor activation and modulation when extracellular Ca^{2+} is >1 mmol/L. Thus, there exists a co-agonist mechanism whereby extracellular Ca^{2+} and L-amino acids are required to maximally activate the CaSR.[37,47] The ECD of the CaSR also possesses allosteric binding sites for anions, including phosphate.[37] They negatively modulate the CaSR by facilitating the inactive state, thereby inhibiting the CaSR and permitting PTH release.[37,48]

CaSR G protein signaling cascades: Upon activation, the CaSR induces signaling cascades (Figure 2) via coupling to G proteins, specifically G alpha subunits such as Gq/11, Gi/0, and G12/13.[49–51] Double knockout of the alpha subunits Gq and G11 in mouse parathyroid leads to elevated levels of both PTH and serum Ca^{2+}, suggesting that loss of Gq/11 signaling impairs the CaSR to feedback inhibit PTH secretion.[49] Stimulation of Gq/11 activates phospholipase C (PLC) which generates the second messengers, inositol 1,4,5, trisphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol 4,5-bisphosphate (PIP_2).[52] DAG activates protein kinase C (PKC) which

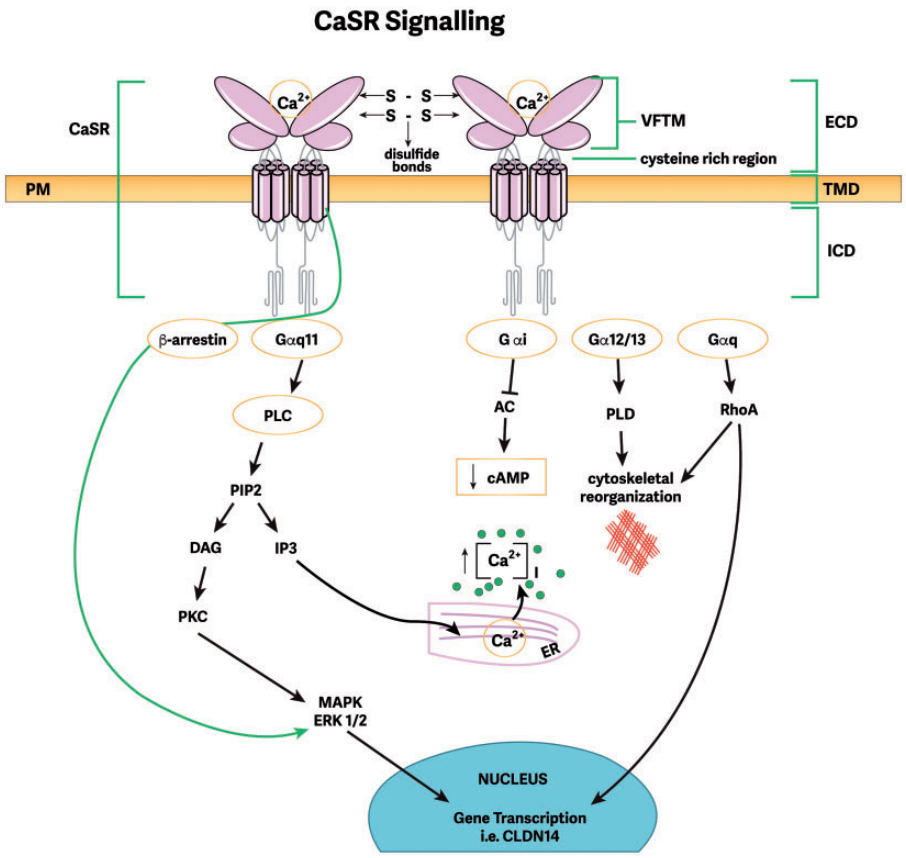

Figure 2. A schematic representation of calcium-sensing receptor (CaSR) signaling within epithelial cells. The CaSR has an extracellular domain (ECD) containing a Venus fly trap module (VFTM) and a cysteine rich region. It has seven transmembrane domains (TMD) and an intracellular domain (ICD). The CaSR forms homodimers by connection of the VFTMs via disulfide bonds. Calcium (Ca^{2+}) binding within the VFTM activates the CaSR leading to signaling through G alpha (Gα) proteins (Gαq, Gαq11, Gαi, Gα12/13) and beta-arrestin (*β*-arrestin). Gαq11 stimulates phospholipase C (PLC)-mediated cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and inositol 1,4,5, trisphosphate (IP3). IP3 induces release of Ca^{2+} from the endoplasmic reticulum (ER), thereby increasing cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$). DAG activates protein kinase C (PKC) resulting in mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases (ERK1/2) mediated gene transcription in the nucleus, and in the TAL specifically *CLDN14*. *β*-arrestin also stimulates MAPK and ERK1/2. Gαi inhibits adenylate cyclase (AC) and decreases cyclic adenosine monophosphate (cAMP). Gα12/13 activates PLD, which affects cytoskeletal reorganization. Gαq activation of Ras homolog family member A (RhoA) also stimulates cytoskeletal reorganization and gene transcription. (A color version of this figure is available in the online journal.)

initiates mitogen-activated protein kinase (MAPK) signaling cascades.[53] Subsequently, MAPKs, including the extracellular signal-regulated kinases ERK1/2 mediates cytosolic signaling and activation of gene transcription.[53,54] The second messenger, IP3, stimulates release of Ca^{2+} from the endoplasmic reticulum thereby increasing cytosolic Ca^{2+} which activates other pathways.[51,55] Additionally, an *in vitro* study using HEK-293 cells showed that CaSR signaling through the Gq alpha protein activates the small GTPase, RhoA, stimulating serum response element-mediated gene expression.[56] Coupling of the CaSR to G12/13 activates the Rho family of small G proteins and phospholipase D, resulting in cytoskeletal reorganization.[50] CaSR signaling through Gi/0 inhibits adenylate cyclase, resulting in a decrease in cAMP.[51] Furthermore, CaSR activation can also initiate G-protein independent signaling through *β*-arrestin, which stimulates ERK1/2 of the MAPK pathway.[57] Overall, the downstream signaling cascades that arise from G-protein-dependent and -independent pathway activation by the CaSR contribute to the control of Ca^{2+} homeostasis through increased cytosolic Ca^{2+}, MAPK, RhoA signaling, and decreased cAMP. These cell events lead to changes in gene transcription and reorganization of the cytoskeleton, although the specific mechanisms affecting target proteins (i.e. transporters or tight junction proteins) important in maintaining serum Ca^{2+} levels are largely unknown. Furthermore, the downstream signaling cascades used by the CaSR may differ between organs, although this remains to be determined.

CaSR signaling in the kidney

Glomerulus: The glomerulus filters blood to form pro-urine. CaSR protein in the glomerulus has been found in some studies, but not others.[5,30,31] In studies that identified CaSR protein in the glomerulus, expression was sparse.[31] In cultured human mesangial cells, CaSR stimulation caused a PLC-mediated increase in intracellular Ca^{2+} via influx through canonical transient receptor potential (TRP) channels TRPC3 and TRPC6.[58] This induced cell proliferation.[58] Furthermore, outside the glomerulus, expression was found in isolated mouse juxtaglomerular cells and calcimimetics decreased cAMP production, which resulted in decreased renin release.[59]

Proximal tubule: The PT is responsible for the majority of water and electrolyte reabsorption, with > 60% of filtered

Ca^{2+} being reabsorbed from this segment through a paracellular pathway.[60] Apical expression of the CaSR has been detected, although at a low level and not consistently in the PT,[26,27,31] suggesting the CaSR could be involved in sensing luminal Ca^{2+}. In a perfusion study of rat PTs, use of the CaSR selective calcimimetic, R-568 or increasing luminal Ca^{2+} concentration, increased fluid reabsorption and sodium/proton exchanger (NHE3)-mediated proton secretion.[61] These effects were not seen in the PT of animals lacking the CaSR.[61] The apically located CaSR mediates Ca^{2+} reabsorption indirectly by increasing NHE3 activity.[61,62] The transcellular flux of sodium, along with water flux, generates the driving force for Ca^{2+} reabsorption via the paracellular route.[63] The specific signaling cascades involved in CaSR activation of downstream effectors that mediate its function in the PT are largely unknown. However, stimulation of the CaSR in the PT-derived opossum kidney cell line by extracellular Ca^{2+} or the CaSR agonist, neomycin, resulted in an increase in cytosolic Ca^{2+} and ERK1/2 activation via the PIP_2–PLC pathway, but not the PKC pathway.[64] Overall, the role of the CaSR in the PT is controversial, as some have not been able to identify expression of the CaSR in the PT at all, or at levels unlikely to be physiologically significant.[5,29–31]

Thick ascending limb of Henle's loop: Approximately 25% of filtered Ca^{2+} is reabsorbed from the TAL through a paracellular pathway. The furosemide sensitive sodium-potassium-chloride-cotransporter 2 (NKCC2) reabsorbs sodium, potassium, and chloride into TAL epithelial cells.[65,66] The subsequent asymmetrical flux of potassium back into the lumen through the renal outer medullary potassium channel (ROMK) and chloride ions basolaterally via the voltage-gated chloride channel Kb (CLCNKB) generates a lumen positive voltage potential critical for driving Ca^{2+} paracellularly.[66,67] This system is regulated by CaSR activity. Mice with a kidney specific deletion of the CaSR have four-fold increased expression of active, phosphorylated-NKCC2,[68] which would increase Ca^{2+} reabsorption through the paracellular pathway. Further the peritubular addition of NPS2143, a CaSR inhibitor, to isolated perfused rat TAL increased Ca^{2+} reabsorption without altering sodium or chloride-flux, or transepithelial voltage.[5] This suggests that the CaSR directly affects the paracellular permeability of the epithelium to Ca^{2+}. Indeed, CaSR activation increases the expression of the tight junction protein, CLDN14, in response to elevated serum Ca^{2+} levels.[20] *Cldn14* transcripts are detectable at higher levels in the TAL compared with other tubular segments[22,69] and increases substantially when animals are fed a high Ca^{2+} diet,[70] made hypercalcemic or given calcimimetics.[20] Expression of the CLDN14 protein is only visible under hypercalcemic conditions, where it is restricted to the outer stripe and cortical TAL segments,[69] where it functions to block paracellular Ca^{2+} reabsorption, resulting in increased urinary Ca^{2+} excretion.[20] The tight junction proteins, CLDN16 and CLDN19 co-localize in the TAL and interact to form a cation-selective pore.[21] CLDN14 attenuates paracellular cation reabsorption through the CLDN16/19 tight junction complex by physically interacting with CLDN16.[22] Moreover, mice with kidney specific knockout of the CaSR had reduced *Cldn14* and increased *Cldn16* expression, further supporting that CaSR activity regulates tight junction protein expression to attenuate Ca^{2+} reabsorption from the TAL.[68] The microRNAs, miR-9 and miR-374 have been implicated in repressing CLDN14 gene transcription via binding the 3′-UTR of CLDN14 mRNA.[22,71] CaSR activation decreases miR-9 and miR-374.[71] Otherwise the CaSR signaling cascades mediating altered Ca^{2+} transport in this segment are largely unknown; however, G-protein mediated signaling through Gq and/or G11 likely contributes. Furthermore, other CaSR-dependent signaling pathways than those described in parathyroid may exist in kidney. Regardless, the CaSR in the basolateral membrane of the TAL senses serum ionized Ca^{2+} and signals to alter urinary Ca^{2+} excretion in response.

Distal convoluted and connecting tubule: Reabsorption of the remaining up to 10% of filtered Ca^{2+} occurs in the DCT and CNT via an active transcellular transport mechanism.[65,72] Luminal Ca^{2+} enters the DCT/CNT cell through the apically expressed TRP vanilloid 5 (TRPV5) channel and is shuttled to the basolateral membrane by calbindin-D_{28K} (CALB28K), where efflux occurs via the plasma membrane calcium ATPase (PMCA) and sodium–calcium exchanger 1.[73] Activation of the basolateral CaSR in a distal tubular cell model (MDCK cells) inhibits transcellular reabsorption of Ca^{2+} by decreasing PMCA activity in a PLC-dependent manner.[74] Some reports also suggest that the CaSR is expressed apically in the DCT and CNT where it colocalizes with TRPV5 at the luminal membrane.[75] Activation of the CaSR in HEK293 cells stimulates TRPV5-mediated Ca^{2+} influx, via a phorbol-12-myristate-13-acetate-insensitive PKC isoform pathway.[75] Mutation of PKC phosphorylation sites on TRPV5 abolished channel activity.[75] Therefore, the CaSR signals through different intracellular pathways depending on cellular localization in this segment (apical vs. basolateral). Based on these observations it is suggested that the basolateral CaSR senses serum Ca^{2+} levels and functions to decrease renal Ca^{2+} reabsorption, whereas apical CaSR senses lumen Ca^{2+} levels and functions to increase reabsorption.

Collecting duct: There is little Ca^{2+} reabsorption from the CD; however, some have reported expression of the CaSR in this segment. An increase in luminal Ca^{2+} decreases tubule permeability to water and increases proton secretion by downregulating aquaporin 2 (AQP2) and increasing H^+-ATPase activity respectively.[76] Luminal Ca^{2+}-mediated polyuria and urinary acidification may protect from renal stone formation. The apically located CaSR in the CD senses the luminal Ca^{2+} concentration, as activation of the CaSR by luminal addition of CaSR agonists decreased AQP2 expression and water permeability, and increased H^+-ATPase activity.[28,76,77] The effects of CaSR activation on AQP2 expression were found to be independent of PKC and ERK, but relies on increased cytosolic Ca^{2+}, calmodulin, and protein kinase A.[77] CaSR protein was also found within purified AQP2 endosomes along with Gq/11, Gi, and two PKC isoforms[76] implicating the CaSR in trafficking of AQP2 to the membrane. Again there is debate as to whether there is CaSR in this segment.

Loupy *et al*.[5] suggest Ca^{2+} sensing in the CD occurs through a different mechanism (i.e. via Gprc6a), as CaSR agonists did not elicit a Ca^{2+} response in microperfused CDs when applied either luminal or basolateral and they could not observe CaSR expression in the CD. However, others have found that 5 mmol/L extracellular Ca^{2+} was not able to elicit an intracellular Ca^{2+} response in HEK-293 cells transfected with Gprc6a.[78] Additionally, maximal activation of Gprc6a, as determined by phosphorylated-ERK levels, required 40 mmol/L extracellular Ca^{2+} compared with 5 mmol/L required to maximally activate the CaSR. Gprc6a seems to have lower affinity for Ca^{2+} compared with the CaSR, and thus the CaSR is likely the main Ca^{2+} sensor in this segment.

Intestinal CaSR signaling

Intestinal Ca^{2+} absorption can occur by both paracellular and transcellular routes, and is strongly regulated by 1, 25-dihydroxyvitamin D. Activation of the apical intestinal CaSR purportedly reduces Ca^{2+} absorption.[79] This is supported by the apical addition of the CaSR agonists cinacalcet and AC-265347 to monolayers of human intestinal (Caco-2) cells, which decreased 1,25-dihydroxyvitamin D-induced Ca^{2+} flux.[79] The specific mechanisms of how CaSR activation affects the 1, 25-dihydroxyvitamin D-dependent intestinal Ca^{2+} absorption are largely unknown. Whether and how intestinal CaSR activation modulates paracellular Ca^{2+} absorption is not clear, although the CaSR has been found to be important in maintenance of the intestinal epithelial barrier integrity.[80] For example, CaSR null mice have reduced colonic transepithelial resistance, greater transepithelial conductance and higher passive transport as measured by the flux of FITC-conjugated dextran.[80] Through pharmacological and physiological methods, the intestinal CaSR has recently been found to affect the transcellular transport of Ca^{2+}.[6] Basolateral but not apical CaSR activation by cinacalcet or higher Ca^{2+} concentration in the duodenum, cecum, and proximal colon decreased apical TRPV6-mediated intestinal Ca^{2+} absorption through the PLC pathway.[6]

In addition to nutrient sensing, maintenance of epithelial barrier integrity, and regulation of Ca^{2+} absorption, the intestinal CaSR modulates water transport in the intestine. Both the apical and basolateral CaSR in the colon are functionally active and modulate water transport there.[36] Consistent with this in isolated perfused rat colonic crypts an increase in luminal or basolateral Ca^{2+} from 0.1 to 2.1 mmol/L or addition of CaSR agonists resulted in an increased intracellular Ca^{2+} response and reduced fluid secretion. The PLC–IP_3 pathway was implicated in mediating the intracellular Ca^{2+} increase, since CaSR activation increased intracellular IP_3 accumulation and a specific inhibitor of PLC, U-73122, prevented the response. An additional study found that both luminal and basolateral CaSR activation in isolated perfused colonic crypts, reduced forskolin-stimulated fluid secretion.[45] Similarly, *ex vivo* perfusion of small intestinal segments with luminal addition of a CaSR agonist, reduced fluid secretion.[81] Overall intestinal epithelia clearly can sense extracellular Ca^{2+} levels and respond to maintain plasma Ca^{2+} but more work is required to fully understand the signaling pathways mediating this and if the paracellular pathway is involved.

Clinical conditions associated with increased CaSR signaling

The maintenance of serum Ca^{2+} levels within a normal range is dependent on the CaSR.[2] Consequently, mutations in the CaSR that alter the affinity for Ca^{2+} 'reset' serum Ca^{2+} levels either upwards or downwards.[82] Low Ca^{2+} levels, or hypocalcemia, is not normally detected by the CaSR, enabling the secretion of PTH and preventing its degradation.[2] A small decrease in serum Ca^{2+} may be corrected entirely through alterations in PTH release. However, activating mutations in the CaSR, bind serum Ca^{2+} and signal, even when serum Ca^{2+} is low, resulting in hypocalcemic syndromes (Table 1). These mutations increase the receptor's affinity for Ca^{2+} inducing a left shift in the dose response curve, such that even lower serum Ca^{2+} levels are sufficient to activate the receptor.[83,84]

Patients with activating CaSR mutations typical display a syndrome referred to as autosomal dominant hypocalcemia type 1 (ADH1). They exhibit mild to moderate low blood Ca^{2+} levels with inappropriately low PTH levels.[84] They can exhibit neuromuscular irritability, seizures, and basal ganglia calcification. Heterozygous mutations are most common and those with homozygous mutations do not appear to exhibit a more severe phenotype. Other gain-of-function CaSR mutations cause Bartter's syndrome type V. This is a more severe phenotype characterized by urinary sodium loss, due to inhibition of sodium reabsorption from the TAL as well as hypocalcemia, hypoparathyroidism, and hypomagnesemia since Mg^{2+} is reabsorbed by a similar mechanism to Ca^{2+} in the TAL.[85]

The CaSR signals by coupling to G-proteins, including Gα11. It is not surprising then that gain-of-function mutations in Gα11, cause hypocalcemia with reduced PTH levels, i.e. ADH type 2 (ADH2; Table 1).[53,86] Mutations in *GNA11* (the Gα11 gene) were identified by screening patients with ADH, but without mutations in the CaSR.[87] Mutations in *GNA11* causing ADH2 increase the affinity of the CaSR for Ca^{2+} similar to CaSR gain of function mutations. Interestingly, patients with ADH2 described thus far have not presented with hypercalciuria, in contrast to 10% of ADH1 patients who exhibit this at presentation. However, ADH2 is less common and the number of patients studied to date is limited.

The CaSR signals in a biased manner.[88] Activating or inactivating mutations in the CaSR act as 'molecular switches' to direct signaling either by increasing intracellular Ca^{2+} or via MAPK signaling. Mutations causing ADH1 appear to preferentially signal via increased Ca^{2+}, in contrast to loss-of-function mutations, which signal mainly through the MAPK pathway.[57] However, exceptions exist, as the R680G activating mutation in the CaSR stimulates the MAPK pathway without altering intracellular Ca^{2+} responses, in contrast to the majority of other activating mutations that bias the CaSR towards Ca^{2+} signaling.

Table 1. Disorders of CaSR signaling.

Syndrome	Gene	Mutations	Clinical phenotype*	References
Hypercalcemic				
FHH1	*CaSR*	I40F, I555T, E297K, 1608 + 3 A > G, G571W, G146D, P798T—(Het) Q459R & I81K (Hom)	Hypercalcemia, hypophosphatemia, hypocalciuira, hyperparathyroidism, hypermagnesemia	98,104–112
FHH2	*GNA11*	F220S, T54M—heterozygous	Hypercalcemia, hypophosphatemia, hyperparathyroidism, hypocalciuria	113,114
FHH3	*APS2*	R15C, R15H and R15L—heterozygous	Hypercalcemia, low bone mineral density, hypocalciuria	115
NSHPT	*CaSR*	P39A, E519X, IVS5 + 1 G > A, 206 G > A—homozygous R185Q (Het)	Hypercalcemia, hypophosphatemia, hypocalciuria, hyperparathyroidism, hypermagnesemia, hypotonia, fractures, osteopenia, bell-shaped ribs, elevated Alk Phos, elevated $1,25(OH)_2D_3$, low $25OHD_3$	93,107,116–123
Hypocalcemic				
Barter syndrome	*CaSR*	L125P, A843E, Y825F—heterozygous	Hypokalemia, met. alkalosis, hypocalcemia, hypoparathyroidism, hyperphosphatemia, hypercalciuria, hypomagnesemia, hyperaldosteronism, nephrocalcinosis, tetany	98,124
ADH1	*CaSR*	L123S, N732S, P221L, T888M, C131S, E767K, C129S, Q681R—heterozygous	Hypocalcemia, hypoparathyroidism, hyperphosphatemia, hypercalciuria, hypomagnesemia, tetany, nephrocalcinosis, nephrolithiasis, elevated $1,25(OH)_2D_3$, low $25OHD_3$	125,126,109,127–132
ADH2	*GNA11*	V340M, R60L—heterozygous	Hypocalcemia, hypoparathyroidism, hyperphosphatemia, tetany	133
OCS/GBD	*FAM111A*	S343del, T338A, D528G, P527T—heterozygous	Hypocalcemia, hypoparathyroidism, hyperphosphatemia, fractures, frontal bossing, large ant. Fontanelle, flat nasal bridge, low set ears, clover leaf skull, hypertelorism, cataracts, thin lone bones and ribs, hypoplasia, shortened limbs, hepatomegaly, oligohydramnios	134–140
KCS	*FAM111A*	R569H, Y511H, S541Y—heterozygous	Hypocalcemia, hypoparathyroidism, hypomagnesemia, hyperphosphatemia, open anterior fontanelle, prominent forehead, macrocephaly, hyperopia, myopia, medullary stenosis, cortical thickening	129,136,137,138

FHH: familial hypocalciuric hypercalcemia; NSHPT: neonatal severe hyperparathyroidism; ADH: autosomal dominant hypocalcemia; OCS: osteocraniosyndostosis; GBD: gracile bone dysplasia; KC: Kenny Caffey syndrome; Het: heterozygous; Hom: homozygous.
• NB there can be significant variability in clinical phenotypes and all findings may not be present.

The treatment for ADH is non-specific and includes active vitamin D and Ca^{2+} supplementation.[82] However, treatment with Ca^{2+} and active vitamin D can cause nephrocalcinosis resulting in impaired renal function. Calcilytics are antagonists of the CaSR that are a possible treatment for ADH. Calcilytics are proposed to cause normocalcemia in ADH patients by negatively modulating the CaSR, suppressing CaSR signaling, and thus stimulating PTH secretion.[89] Importantly, calcilytics have yet to show broad efficacy likely due to the heterogeneity of a treatment response caused by large variability in *CASR* mutations causing ADH and thus differences in drug-binding with mutant CaSR receptors.

Clinical conditions associated with decreased CaSR signaling

In response to increased serum Ca^{2+} levels, CaSR signaling decreases PTH secretion, increases renal Ca^{2+} excretion, and decreases intestinal Ca^{2+} absorption.[2] In contrast to gain-of-function CaSR mutations, loss-of-function mutations decrease the set-point for PTH secretion, primarily by reducing the receptor's affinity for agonists or failing to activate G-coupled proteins.[53,83] Heterozygous inactivating mutations in the CaSR cause familial hypocalciuric hypercalcemia type 1 (FHH1), a disease that typically presents with mild to moderate symptoms (Table 1).[82] Homozygous inactivating mutations, however, produce a severe hypercalcemic disorder with high PTH levels, called neonatal severe hyperparathyroidism (NSHPT).[82] This gene dosage effect, found commonly with many inactivating mutations of the CaSR, causes PTH levels to increase more than five-fold, resulting in hyperplasia of the parathyroid glands.[90] Affected neonates also have demineralized bones and rib fractures from chronically elevated PTH, which can cause respiratory difficulties and even death.[82]

Approximately 65% of FHH patients have FHH1, which is due to inactivating mutations in the CaSR.[91] The majority of FHH1 CaSR mutations (>50%) are missense mutations in the large ECD, which reduce the receptor's affinity for agonists.[92] Patients, however, generally only have mild hypercalcemia, normal PTH, and mild hypermagnesemia. They are usually heterozygous and thus the normal functioning CaSR typically limits the clinical phenotype.[91]

However, not all patients with heterozygous mutations have mild disease.[90] Disease severity appears to increase when the mutation is paternally transmitted or *de novo*. This is postulated to occur as a fetus with a FHH mutation that is gestated in a normocalcemic mother is more likely to develop secondary hyperparathyroidism. However, symptoms typically improve over time as the fetus is no longer impacted by the maternal Ca^{2+} homeostatic system.[93] These children also display some degree of bone disease which can be treated with parathyroidectomy, improving their disease to an asymptomatic state. FHH mutations typically demonstrate CaSR signaling bias towards MAPK pathway activation; however, bias towards intracellular Ca^{2+} mobilization has also been seen.[57,88]

In parallel to ADH2, FHH2 is caused by inactivating mutations of *GNA11* and thus alterations in the Gq/11 protein (Table 1). *GNA11* mutations have been found in more than 10% of FHH patients without CaSR mutations.[87] Heterozygous mutants (e.g. R187Q) exhibit a dominant negative effect as the abnormal receptor interferes with the function of the normal receptor.[93] It is postulated that this may be due to the removal of functional G proteins, limiting the wildtype CaSR ability to signal. FHH2 mutations typically display a modest decrease in affinity for Ca^{2+}, and appear to occur preferentially in the GTPase domain of the small G-coupled protein. Patients typically have mild alterations in serum Ca^{2+} if any at all.

FHH3 is caused by inactivating mutations of the *AP2S1* gene (Table 1). This disease is characterized by high levels of serum Ca^{2+} and PTH as well as hypophosphatemia.[91] Plasma membrane CaSR expression is regulated by internalization and insertion, the former occurring via clathrin-mediated endocytosis.[94] AP2σ, the protein encoded by *AP2S1*, binds the CaSR directly initiating endocytosis.[11] *AP2S1* mutations causing FHH3 interfere with binding to this motif and consequently impair CaSR endocytosis.[95] Specifically, missense mutations that affect Arg15, which is known to form contacts with dileucine-based motifs of clathrin-coated vesicle cargo proteins cause FHH3.[91,96] More than 20% of patients with FHH without a CaSR mutation have AP2S1 mutations.[91] These mutants cause delayed CaSR internalization which increases CaSR plasma membrane expression, yet also hypercalcemia.[94,97] This seemingly paradoxical relationship is hypothesized to be due to a decrease in sensitivity of the CaSR to Ca^{2+} and reductions in Ca^{2+}-mediated phosphorylation of the receptor in response to increasing Ca^{2+} levels. It is important to note that AP2σ mutants hinder, but do not abolish CaSR internalization, explaining the relatively mild phenotype of FHH3 compared with NSHPT, which is caused by homozygous mutations in the CaSR.[2]

An emerging treatment for hypercalcemic disorders is calcimimetics, which are positive modulators of the CaSR.[89] Calcimimetics interact with the transmembrane domain of the CaSR, increasing the receptor's affinity for Ca^{2+}.[82] Thus, calcimimetics are an emerging therapy which might be useful to lower serum Ca^{2+} levels, assuming that the mutant CaSR is responsive.[82]

FAM111A a potential modifier of CaSR signaling

Dominant missense mutations in *FAM111A* are the underlying cause of Kenny Caffey syndrome type 2 (KCS2), gracile bone dysplasia (GBD), and perinatally lethal osteocraniostenosis (OCS; Table 1).[98] Patients with these diseases display a phenotype that includes hypocalcemia, hypoparathyroidism, medullary stenosis of tubular bones, and short stature. Thus, mutations in FAM111A result in a phenotype, which in part resembles gain-of-function mutations in the CaSR. This similarity in phenotype begs the question as to how FAM111A might affect CaSR signaling, including across epithelia. Unfortunately, *FAM111A* is a poorly characterized gene; however, it is predicted to be a serine protease. Its C-terminus has a trypsin-like domain containing the catalytic triad, His385, Aps439, and Ser541, which is characteristic of the S1 family of serine proteases.[99–101] FAM111A appears capable of auto-cleavage *in vitro*. FAM111A localizes in the nucleus and is also found to be a proliferating cell nuclear antigen (PCNA)—interacting protein that works to mitigate the effects of protein obstacles on replication forks that would otherwise block replication. As such, it could act as a transcription modifier, interacting with PCNA through its PCNA-interacting peptide box, and thus affect CaSR transcription and activity.[102]

All disease-causing mutations reported thus far are in the serine protease domain of FAM111A, inferring alteration in this function result in the CaSR activation like phenotype. Gain-of-function mutations in FAM111A amplify protease activity, antagonizing chromatin-associated processes including DNA replication and transcription by displacing key effectors, which may result in rapid caspase-dependent apoptosis.[98] Some disease-causing mutations (e.g. R569H, Y511H) decrease the amount of full-length FAM111A protein and increase levels of autocleavage fragments when expressed in cell culture, inferring they are activating mutations.[99] Alternatively, rather than stimulating CaSR transcription, parathyroid development could be impaired by hyperactive FAM111A inducing apoptosis in this gland thereby causing KCS2, GBD, or OCS. Another possibility is that disease-causing activating *FAM111A* mutations increase protease activity thereby preventing the downregulation of CaSR expression and activity via increased degradation of key players involved in CaSR desensitization and internalization (e.g. β-arrestins and G protein receptor kinases).[78,103] Although highly speculative, this may explain why *FAM111A* mutations cause phenotypes resembling a gain of function mutation in the CaSR.

Summary/conclusions

The CaSR plays an essential role in Ca^{2+} homeostasis by sensing extracellular Ca^{2+} levels and signaling to maintain the serum Ca^{2+} concentration within a narrow physiological window. It is located in organs that are essential in regulating the body's Ca^{2+} balance, such as the parathyroid, kidney, and intestine. In the kidney, the CaSR is

predominately expressed in the basolateral membrane of the TAL and responds to changes in blood Ca^{2+} levels by regulating tight junction proteins, thereby affecting urinary Ca^{2+} excretion. The expression and function of the CaSR in other nephron segments are still debated, and may not be physiologically relevant to the maintenance of Ca^{2+} homeostasis. The CaSR is expressed along the intestine and functions in modulating Ca^{2+} and water absorption. It is unknown whether and how the intestinal CaSR influences paracellular Ca^{2+} permeability in response to changes in the blood Ca^{2+} concentration. However, the effect of CaSR activation on the transcellular pathway of Ca^{2+} absorption is beginning to be delineated. Overall in epithelia upon activation, the CaSR signals through a variety of G-protein-dependent and -independent pathways, with the signaling pathway initiated potentially differing depending on the organ or cellular localization of the CaSR. It is largely unknown how CaSR signaling cascades specifically regulate downstream effectors of Ca^{2+} transport. Perturbations in CaSR activity cause a range of diseases with altered serum Ca^{2+} levels, such as FHH and ADH. This highlights the importance of understanding CaSR signaling during the maintenance of Ca^{2+} homeostasis in order to help restore normal serum Ca^{2+} levels. Indeed, the recent advances in understanding CaSR signaling have led to the targeting of the CaSR with calcimimetic and calcilytic drugs to help maintain systemic Ca^{2+} homeostasis.

AUTHORS' CONTRIBUTIONS

RSGT and CHLL wrote the first draft of the manuscript. RSGT, CHLL, RTA, and HD reviewed and edited the manuscript.

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ORCID iDs

Rebecca Siu Ga Tan https://orcid.org/0000-0002-0535-1446
R Todd Alexander https://orcid.org/0000-0001-7396-7894

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