

Gastrointestinal and renal regulation of calcium metabolism

Ayu Agbecha *

Department of Chemical pathology, Federal Medical Center Makurdi, Nigeria.

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Abstract

Calcium is critical to human physiology for the function of excitable tissues (e.g. neuromuscular function) and is also needed for skeletal mineralization. The maintenance of a constant free ionized calcium concentration is biologically important for these functions. Regulation of calcium levels is a pre-requisite for its maintenance. In this article, calcium homeostasis is reviewed in respect to the critical organs involved; gastrointestinal tract, and the kidneys. Also reviewed is the calcium sensing receptor (CaSR) which serve as a calcium regulator (calciostat) in calciotropic tissues and regulates other biological functions in non-calciotropic tissues. The purpose of this review is to discuss the normal regulation of calcium metabolism in order to provide the clinician with basic information for the diagnosis and therapeutic management of calcium metabolism disorders as well as related pathologies.

Keywords: Calcium; Gastrointestinal; Renal; calcium sensing receptor

1. Introduction

Calcium is an essential ion within the human body. The maintenance of a constant free ionized calcium concentration is biologically important for the function of excitable tissues [1]. Abnormalities in serum calcium values may have profound effects on neurological, gastrointestinal and renal function. Normal calcium concentrations are maintained via tightly regulated ion transport by the kidneys, and intestinal tract [2]. Regulated calcium ion transport is mainly mediated by parathyroid hormone (PTH) and active vitamin D (calcitriol). Changes in calcium transport resulting in movement into or out of the extracellular fluid leads to hypercalcaemia and hypocalcaemia, respectively [3]. The calcium-sensing receptor (CaSR) ubiquitously expressed in tissues and primarily recognized as the molecular sensor of free ionized calcium is the master regulator (calciostat) of systemic calcium metabolism. Other than calcium regulation, the CaSR play vital roles in several physiologic and pathologic processes, making the receptor a therapeutic target in the development of emerging drugs. In this article the mechanisms responsible for calcium metabolism and disorders is reviewed. Understanding the physiology of calcium homeostasis informs the physician about the clinical approaches in regards to the diagnosis and management of patients with calcium disorders and related pathologies.

2. Functions of calcium

Calcium is required in the body for many vital intracellular and extracellular functions including skeletal support [2]. Ionized calcium (Ca^{2+}) is required for enzymatic reactions, membrane transport and stability, blood coagulation, nerve conduction, neuromuscular transmission, muscle contraction, vascular smooth muscle tone, hormone secretion, bone remodeling (formation/resorption), control of hepatic glycogen metabolism, cell growth, development and proliferation [4]. Intracellular calcium ion could be termed a universal ionic messenger that regulates several cellular responses by the interaction of many agonists with the CaSR at the cell surface of tissues [5]. Extracellular fluid (ECF)

*Corresponding author: Ayu Agbecha
Department of Chemical pathology, Federal Medical Center Makurdi, Nigeria.

Ca^{2+} the major agonist of CaSR regulates cellular processes in many organs including the parathyroid gland, kidney, and thyroid C cells by binding to this cell membrane receptor [6]

3. Daily calcium balance in the body

Calcium balance is a complex process involving bone, intestinal absorption of dietary calcium, and renal excretion of calcium (figure 1). In healthy adults, approximately 800–1000 mg of calcium is ingested daily. This amount varies depending on the amount of dairy product consumed. When 1000 mg of calcium is ingested in the diet, approximately 900 mg is excreted in the feces and 100 mg in the urine [7]. Approximately 350mg of the usual 1000mg dietary calcium intake is absorbed by the intestine, and calcium loss by way of intestinal secretions is approximately 250 mg/day. Therefore, a net absorption of calcium is approximately 100 mg/day (20%) [8]. Although serum calcium levels can be maintained in the normal range by bone resorption, dietary intake is the only source by which the body can replenish stores of calcium in bone.

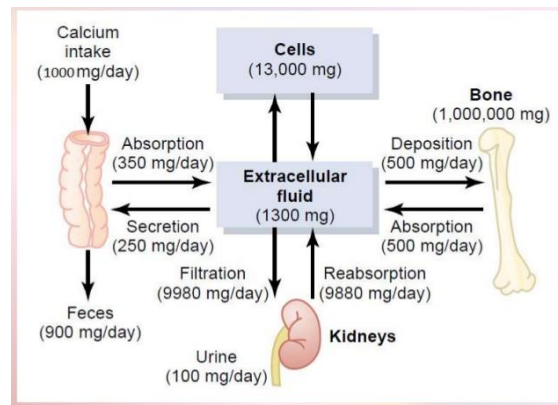


Figure 1 Daily balance between intake and excretion of calcium in the body

4. Calcium homeostasis

Calcium homeostasis refers to the maintenance of a constant concentration of Ca^{2+} in the extracellular fluid. It involves the maintenance of Ca^{2+} balance between the extracellular and intracellular fluid compartments. Because plasma Ca^{2+} concentration $[\text{Ca}^{2+}]$ rapidly equilibrates with the extracellular fluid, ECF $[\text{Ca}^{2+}]$ is kept constant by keeping the plasma $[\text{Ca}^{2+}]$ constant. Since intracellular fluid ICF $[\text{Ca}^{2+}]$ depends indirectly on plasma $[\text{Ca}^{2+}]$, both ECF and ICF $[\text{Ca}^{2+}]$ are linked to plasma $[\text{Ca}^{2+}]$. Thus maintaining constant plasma $[\text{Ca}^{2+}]$ is important in the execution of ECF and ICF calcium functions earlier stated [9].

Calcium homeostasis is controlled by bidirectional calcium fluxes, occurring at the levels of intestine, bone, and kidney [10]. Fluxes of calcium between the small intestine (calcium absorption site), bone (calcium storage site), and the kidney (site for elimination of the absorbed calcium) are highly controlled by numerous transport mechanisms, hormones, and interconnected feedback loops. Since calcium is a highly reactive ion which has high propensity to form microcrystals in fluids and tissues, its homeostatic regulation is an absolute requirement in preventing undesired bio-mineralization in tissues [10].

At the intestinal, bone and kidney levels, calciotropic peptides (PTH, calcitonin), vitamin D metabolites, and a variety of local or systemic factors are capable of modulating different calcium fluxes in these tissues. Any alterations in the secretion of these factors or pharmacological over dosage can lead to an imbalance in calcium fluxes resulting to disorders of calcium metabolism.

5. Gastrointestinal regulation of plasma calcium levels

Calcium is absorbed almost exclusively within the duodenum, jejunum, and ileum. Each of these intestinal segments has a high absorptive capacity for calcium, with their relative calcium absorption being dependent on the length of each respective intestinal segment and the transit time of the food bolus. Calcium usually is freed from complexes in the diet during digestion and is released in a soluble and typically ionized form for absorption. However, small-molecular-weight complexes such as calcium oxalate and calcium carbonate can be absorbed intact. Fractional calcium absorption

(absorptive efficiency) generally varies approximately and inversely with the logarithm of intake, but the absolute quantity of calcium absorbed increases with intake [11].

There are two routes for calcium absorption across the intestinal epithelium (Figure 2): the paracellular pathway (i.e., between the enterocytes) and the transcellular route (i.e., through the enterocytes) [7]. When calcium intake is low, calcium is mainly absorbed by active (transcellular) transport, during high intakes; an increasing proportion of calcium is absorbed by simple (paracellular) diffusion. Unabsorbed dietary calcium put together with the unabsorbed calcium in secreted digestive juice forms total fecal calcium [11].

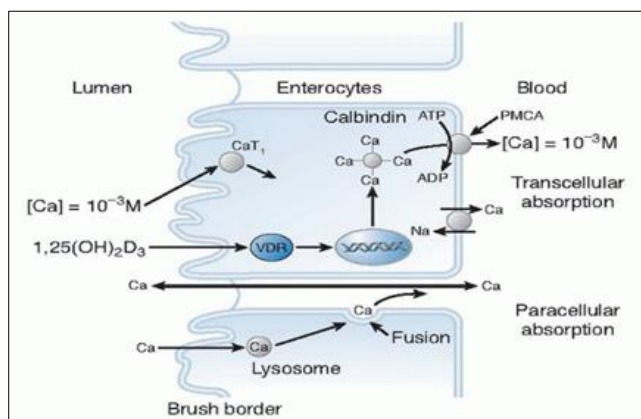


Figure 2 Intestinal pathways for calcium absorption across the intestinal epithelium

The transcellular pathway of the intestinal Ca^{2+} absorption comprises 3 steps (Figure 2). First, the entrance of Ca^{2+} across the brush border membranes (BBM) of enterocytes through epithelial Ca^{2+} channels: transient receptor potential vanilloid 6 (TRPV6) or calcium transporter 1 (CaT1) and TRPV5 or CaT2. Second, the movement of Ca^{2+} from the BBM to the basolateral membranes (BLM) by binding to proteins with high Ca^{2+} affinity (calbindins or CB). Third, intestinal Ca^{2+} extrusion into the blood occurs via plasma membrane Ca^{2+} -ATPase (PMCA1b or Ca^{2+} pump) and the $\text{Na}^+/\text{Ca}^{2+}$ exchanger I (NCX1) [12]. The mechanisms of transcellular intestinal calcium transport include; (1) Calcium movement down its concentration gradient via calcium channel or calcium transporter into the apical section of the microvillae. Because the intestinal concentration of calcium usually is 10^{-3} mol and the intracellular calcium concentration is 10^{-6} mol, a large concentration gradient favors the passive movement of calcium (Figure 2). Calcium is rapidly and reversibly bound to the calmodulin-actin-myosin I complex. Calcium may then move to the basolateral area of the cell by microvesicular transport, or diffusion. (2) As the calmodulin complex becomes saturated with calcium, the concentration gradient for the movement of calcium into the microvillae is unfavorable, and as a result slows calcium absorption. (3) Under the influence of $1,25(\text{OH})_2\text{D}_3$ (calcitriol), intestinal epithelial cells calbindin levels is increased (Figure 2). (4) Calcium binds to calbindin, thereby unloading the Ca-calmodulin complexes, and as a result removes calcium from the microvillae region. Decrease in calcium levels again favors the movement of calcium into the microvillae. As the calbindin-Ca complex dissociates, free intracellular calcium is actively extruded from the cell via Ca-adenosine triphosphatase (ATPase) or NCX1. Calcitriol may increase the synthesis of plasma membrane Ca-ATPase, thereby aiding active extrusion of calcium into the lamina propria.

The movement of molecules and ions via the paracellular pathway is regulated by the tight junctions, which are specialized membrane domains mostly positioned in the apical region of enterocytes (Figure 2). Tight junctions are intercellular structures where plasma membranes of adjacent cells have very close contact. These junctions are composed of transmembrane proteins, cytoskeleton components and cytoplasmic plaques [13]. The transmembrane proteins of tight junction structures are synthesized in the adjacent cells and they include occludin (Ocln) and claudins (Cldns). These proteins close intercellular junctions and restrict the free movement of materials through the paracellular space. Cytoplasmic plaques, such as zona occludens (ZO) proteins contain a binding domain for transmembrane proteins, and are associated with the structure and formation of the tight junctions, and possibly with the paracellular ion transport [14]. The paracellular pathway is passive and it is the predominant means of calcium absorption when the luminal concentration of calcium is high. This is a non-saturable pathway and can account for one half to two thirds of total intestinal calcium absorption.

There is an interaction between the transcellular and paracellular pathways involved in intestinal Ca^{2+} absorption. Data indicate that the expression of paracellular tight junction genes is regulated by transcellular calbindin proteins, which

suggests that active and passive Ca^{2+} transport pathways may function cooperatively [15]. The paracellular absorptive route may be indirectly influenced by $1,25(\text{OH})_2\text{D}_3$ predominantly in the jejunum and ileum by altering the structure of intercellular tight junctions via the activation of protein kinase C (PKC), making the tight junction more permeable to the movement of calcium [16]. However, $1,25(\text{OH})_2\text{D}_3$ primarily controls the transcellular absorption of calcium. Thus, calcitriol stimulates the transcellular and paracellular absorptive pathways by inducing the expression of genes and proteins involved in Ca^{2+} transport and modifying the permeability of tight junctions [17].

5.1 Factors that regulates intestinal absorption of calcium

Both transcellular and paracellular intestinal Ca^{2+} absorption is regulated by hormones and certain physiologic conditions: PTH, thyroid, growth hormones/ IGF-1, estrogen, prolactin, age, pregnancy, lactation [17]. Hormones may influence intestinal calcium absorption by modulating the actions of calcitriol. PTH regulates the conversion of $25\text{-}(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$ and other metabolites of vitamin D. Thyroid hormones enhance the genomic actions of calcitriol whereas glucocorticoids inhibit the transcellular pathway by affecting the expression of Ca^{2+} transporting proteins. Fibroblast growth factor inhibits the intestinal Ca^{2+} absorption antagonizing $1,25(\text{OH})_2\text{D}$ action. Growth hormone enhances intestinal Ca^{2+} absorption through vitamin D dependent and independent mechanisms [17]. Intestinal Ca^{2+} absorption varies according to the physiologic state of the individual. When Ca^{2+} needs are high especially in states of low dietary Ca^{2+} , the intestinal Ca^{2+} absorption turns to be more efficient. Growth, pregnancy, lactation, dietary Ca^{2+} deficiency and high physical activity increases Ca^{2+} demands promoting intestinal absorption of Ca^{2+} . During pregnancy, intestinal Ca^{2+} absorption is higher before conception or after delivery. This increment occurs in early-to mid pregnancy and precedes the increased Ca^{2+} demand from the fetus for skeletal growth. Increment in intestinal Ca^{2+} absorption during pregnancy may be due to increased serum calcitriol with little alteration in PTH and calcitonin concentrations [18]. As a consequence of aging, intestinal Ca^{2+} absorption decreases; malabsorption of Ca^{2+} begins approximately at or between 65 and 70 years [19]. In postmenopausal women, Ca^{2+} malabsorption begins earlier, but is however reversible with estrogen therapy [20]. In During aging, reasons for decreased intestinal absorption of Ca^{2+} in relation to vitamin D metabolism include: (1) Decreased renal synthesis of calcitriol by aged kidney; (2) Intestinal resistance to circulating calcitriol; (3) Decreased intestinal VDR; (4) Decreased skin synthesis of vitamin D; and (5) Substrate deficiency of vitamin D [21]. It has been demonstrated that intestinal Ca^{2+} absorption is also affected by oxidative stress [22]. Gastrointestinal tract is an important source of reactive oxygen species (ROS). Despite the protective barrier provided by intestinal mucosa and its adequately-distributed microbiota, digestion-end products and pathogens can trigger inflammatory response which favors oxidative stress. Consequently, various gastrointestinal pathologies such as GI ulcers, celiac disease, GI tumors and inflammatory bowel disease are associated with oxidative stress [23]. After birth, the maternal mammalian gland secretes an elevated amount of Ca^{2+} , which could reach up to 1000 mg/day of milk Ca^{2+} . In order to provide an extra Ca^{2+} for milk production during lactation, the osteoclast-mediated bone resorption and intestinal Ca^{2+} absorption is increased. The specific hormone responsible for milk calcium secretion during lactation remains uncertain, evidence shows that prolactin (PRL) regulates lactation. Charoenphandhu *et al* proposed that PRL stimulates intestinal Ca^{2+} absorption [24]. Intestinal calcium absorption is also influenced by other pathological conditions like morbid obesity, bariatric surgery, diabetes, hypercalciurias, Turner syndrome, and thalassemia. [17]

6. Renal regulation of plasma calcium levels

The kidneys are the major regulators of calcium homeostasis, confirmed by complex dysregulation of mineral metabolism (mineral and bone disorders) associated with chronic kidney disease. In the kidney, the only source of calcium reaching the tubules is ultrafiltrated calcium, consisting of Ca^{2+} and other calcium-containing salts filtered through the glomerulus. Calcium in glomerular filtrate represents approx. 50% of the total plasma calcium, but practically impossible to be precisely measured in clinical settings [25]. This constitutes a major limitation when evaluating the fractional excretion of calcium. Absent secretion or back leak of calcium contributes to the calcium delivered to the tubular system. Consequently, the glomerular filtrate calcium represents the major contributor of calcium reaching the proximal tubule (PT). In certain circumstances the tubular reabsorption system may be overwhelmed by the filtrated load, as seen in primary hyperparathyroidism or in vitamin D intoxication. In these two conditions, calcium reabsorption machinery of the kidney is maximally stimulated but unable to counterbalance the filtered calcium load, leading to hypercalciuria [25].

Along the tubular system, complex transepithelial transport mechanisms allow a highly regulated reabsorption of approx. 98% of filtrated calcium; implying that the remaining amount of calcium excreted in the urine ranges from 1 to 2% (100 to 200 mg) per 24 hours in apparently healthy adults (figure 3). Calcium is filtered at the glomerulus, with the ultrafilterable fraction of plasma calcium first entering the proximal tubule (PT). Within the proximal convoluted tubule (PCT) and the proximal straight tubule (PST), 60%–70% of the filtered calcium is reabsorbed. Notably no calcium

reabsorption occurs within the thin segment of the ascending loop of Henle. The cortical segments of the loop of Henle reabsorb about 20% of the initially filtered load of calcium. Approximately 10% of the filtered calcium is reabsorbed in the distal convoluted tubule (DCT), with another 3%–10% of filtered calcium reabsorbed in the connecting tubule (CNT).

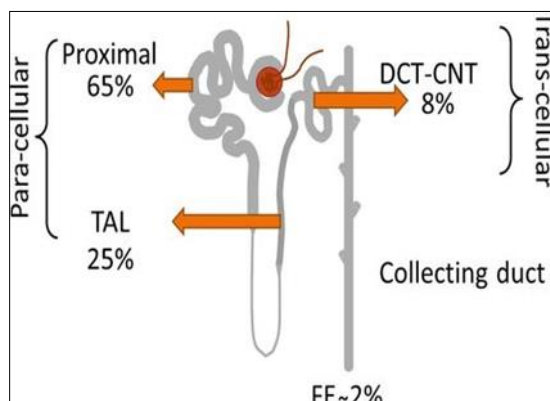
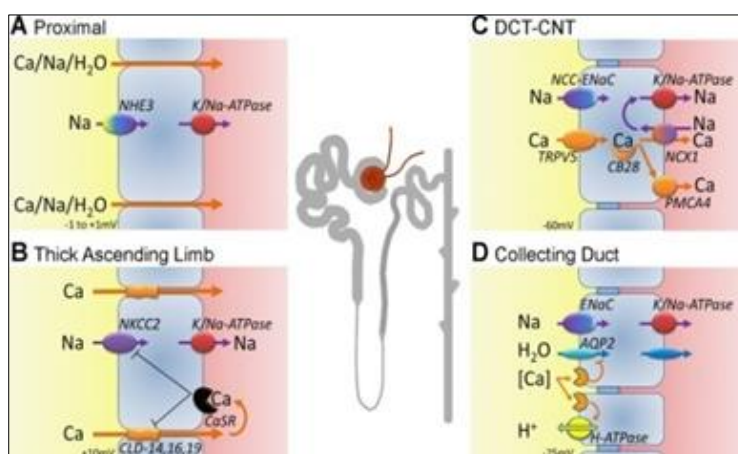


Figure 3 Renal calcium reabsorption

Two major transepithelial calcium transport pathways have been described along the tubules of the kidneys: paracellular and transcellular (Figure 4). Paracellular pathways are dependent on transepithelial electrochemical gradients and can be regulated by specialized paracellular proteins referred as the claudins. The transcellular transport of calcium across the tight tubular epithelium occurs via a three-step process: luminal apical entry, transcytoplasmic transport, and basolateral extrusion. The driving force is mainly provided by basolateral calcium or Na-K-ATPases [25].



sodium/proton exchanger 3 (NHE3), sodium-potassium (Na-K-ATPase), claudin (CLD)16, 19, and 14, sodium/potassium/chloride cotransporter (NKCC2), calcium-sensing receptor (CaSR), distal convoluted-connecting tubule (DCT-CNT), transient receptor potential vanilloid 5 (TRPV5) channels, calbindin D-28k (CB28), sodium/calcium exchanger (NCX1), plasma membrane calcium-ATPase (PMCA4), epithelial sodium channel (ENaC), aquaporin-2 (AQP2),

Figure 4 Detailed renal calcium reabsorption per segment

Proximal tubular calcium is reabsorbed paracellularly, mainly by passive diffusion and solvent drag (figure 4A). The passive paracellular pathways partially driven by the activity of the apical brush border sodium/proton exchanger 3 (NHE3), and the basolateral Na-K-ATPase pumps, account for approximately 80% of calcium reabsorption in this segment of the nephron. The transcellular pathway which enhances a small but significant component of active calcium transport is observed in the PTs. The active transport of calcium proceeds in a two-step process, with calcium entry from the tubular fluid across the apical membrane driven by the activity of NHE3, and calcium exit into blood circulation via the basolateral membrane enhanced by the Na-K-ATPase pumps. This active transport is generally considered to constitute 10%–15% of total proximal tubule calcium reabsorption and mainly regulated by PTH and calcitonin. NHE3 activity and expression are regulated and inhibited by PTH [25]. In the PT, 1α -hydroxylase activity is inhibited by the activation of apical CaSR in the presence of high luminal Ca^{2+} with the subsequent reduction in $1,25(OH)_2D$ synthesis. Thus CaSR tightly controls circulating $1,25(OH)_2D$ levels at the level of its synthesis in the PT. Circulating $1,25(OH)_2D$ enhances intestinal and DCT absorption of calcium. $1,25(OH)_2D_3$, PTH, and dietary phosphate modulates CaSR gene

expression in the PT, suggesting the existence of a local feedback loop for the regulation of extracellular Ca^{2+} and Pi excretion independent of systemic changes in calciotropic hormones [26].

About 20–25% of the filtered calcium is reabsorbed in the thick ascending loop of Henle (TAL), largely by the cortical (CTAL) and, to a lesser extent; by the medullary thick ascending limb (MTAL) via the transcellular and paracellular routes (figure 4B). In the TAL, the bulk of divalent cation (Ca^{2+} and Mg^{2+}) reabsorption proceeds via the paracellular pathway which relies on transtubular electrochemical driving force. The transtubular electrochemical driving force is generated by the rate and extent of Na^+ reabsorption, mediated by the apical NKCC2, renal outer medullary potassium K^+ (ROMK) channel and the basolateral Na^+ - K^+ -ATPase. Apical NKCC2 and ROMK channel generates the “driving force” for paracellular cation transport [27]. While NaCl reabsorption via NKCC2 is electroneutral (NKCC2 translocates 1 Na^+ , 1 K^+ , and 2 Cl^- ions from the lumen into the cell), apical K^+ represents the rate-limiting step of this process and K^+ ions back-diffuse into the lumen through the ROMK channels [28]. Na^+ and Cl^- accumulated inside the cell are then transported into the bloodstream via basolateral Na^+ - K^+ -ATPase and Cl^- channels, respectively. Overall, these processes yield a net cellular reabsorption of NaCl and the generation of a lumen-positive transepithelial potential difference, which drives non-selective cation reabsorption (largely Ca^{2+} and Mg^{2+} , Na^+) via the paracellular route [28]. Calcium is also reabsorbed by specialized and controlled paracellular pathways involving claudins. The tight junction in the TAL expresses several claudins, including claudin-14, claudin-16, and claudin-19. Normal expression of claudin-16 and claudin-19 is required for normal absorption of divalent cations (Ca^{2+} and Mg^{2+}) in this tubular segment. Calciotropic hormones, such as PTH and calcitonin, stimulate active cellular calcium absorption in the CTAL. Studies have demonstrated that CaSR activation inhibits PTH-stimulated apical Ca^{2+} entry, possibly via protein kinase A and C signaling, suggesting that calciotropic hormone induced calcium transport is mediated by the CaSR [29]. Calcium transport in the TAL is influenced by the basolateral CaSR , where it directly controls both paracellular and transcellular NaCl and divalent cation transport. Basolateral/interstitial increases in plasma Ca^{2+} or Mg^{2+} concentrations diminish their own reabsorption, via the activation of basolateral CaSR which subsequently reduces NKCC2 activity and directly modulates paracellular calcium permeability [26]. Rising interstitial calcium concentrations activate the basolateral CaSR , which reduces NKCC2 activity and claudin expression, directly modulating paracellular calcium permeability. Activation of the basolateral CaSR during hypercalcemia also inhibits ROMK channels, which contribute to the recycling of K^+ into the lumen of the TAL [26]. This action of hypercalcemia limits the rate of Na^+ - K^+ - 2Cl^- co-transport by reducing the availability of luminal K^+ . Thus the greater the hypercalcemia, the more inhibition of ROMK, NKCC2 and the faster the dissipation of lumen-positive transepithelial voltage. Overall, CaSR activation abrogates paracellular Na^+ , Ca^{2+} , and Mg^{2+} transport. Inhibition of NKCC2 activity, by certain medications or pathologic conditions decreases the transepithelial voltage, thus diminishing passive calcium absorption [29]. Proximal tubular calcium is reabsorbed paracellularly mainly by passive diffusion and solvent drag (figure 4A). The passive paracellular pathways, partially driven by the activity of the apical brush border sodium/proton exchanger 3 (NHE3), and the basolateral Na^+ - K^+ -ATPase pumps, account for approximately 80% of calcium reabsorption in this segment of the nephron.

In contrast with the PT and TAL, the distal convoluted tubule and connecting tubule (DCT-CNT) reabsorbs calcium exclusively via the transcellular route (figure 4C). The DCT-CNT absorbs 5%–10% of the filtered calcium. In this renal segment, calcium reabsorption is inversely related to Na^+ transport. The transepithelial potential difference (electrochemical gradient) is against Ca^{2+} reabsorption and the paracellular permeability of Ca^{2+} ions is very low. Ca^{2+} reabsorption is active, with sodium reabsorption providing the driving force for calcium transport. Sodium enters the cell via NCC in the first segment of DCT1 or via the epithelial sodium channel (ENaC) in the second segment of DCT2/CNT and exits via the basolateral Na^+ - K^+ -ATPase generating the driving force. Figure 4C is a cell model of the three-step process of transcellular calcium transport regulated by PTH and $1,25(\text{OH})_2\text{D}$. The first step requires calcium influx across the apical membrane and mediated by transient receptor potential vanilloid 5 (TRPV 5) protein. The second step is the diffusion of calcium via the cytosol toward the basolateral membrane: calbindin-D28k binds intracellular calcium transported via TRPV 5 and shuttles it from the cytosol toward the basolateral membrane. The final step involves the extrusion of calcium ions at basolateral membrane via NCX1 and the plasma membrane calcium-ATPase 1b (PMCA1b). The CaSR is present on the basolateral cell membrane and also expressed in a punctuate pattern on apical cell surfaces in DCT. Colocalization of CaSR with TRPV5 at the apical membrane and in subapical vesicles of DCT and CNT cells has been demonstrated [30]. There is evidence that CaSR controls apical Ca^{2+} influx and/or basolateral exit in the DCT-CNT [31].

No calcium reabsorption takes place in the collecting duct-CD (figure 4D), which totally depends on the calcium load delivered by the CNT. However, apical CaSR sense urine calcium concentration which signals the suppression of apical aquaporin-2 (AQP2) and activation of proton (H^+) -ATPase pump. This leads to inhibition of water reabsorption and stimulation of urine acidification, decreasing the risk of stone formation. The CD determines the final concentration of

calcium in urine. By virtue of this, the CD is particularly exposed to the risk of calcium salt precipitation. Two protective mechanisms against calcium salt precipitation have been identified in this segment: acidification and dilution [25].

6.1 Factors that alter renal regulation of calcium

As the major regulatory organ of calcium homeostasis many physiologic, pharmacologic, and pathologic factors influence plasma calcium levels via the kidneys (Table 1) [7].

Table 1 Factors that alter renal regulation of calcium

Increase calcium absorption	Decrease calcium absorption
Hyperparathyroidism	Hypoparathyroidism
High calcitriol	Low calcitriol levels
Hypocalcemia	Hypercalcemia
Volume contraction	ECF expansion
Metabolic alkalosis	Metabolic acidosis
Thiazides diuretics	Loop diuretics

Hypercalcemia is associated with an increase in urinary calcium excretion as a consequence of an increase in the filtered load and a decrease in the tubular reabsorption of calcium. Although hypercalcemia can decrease GFR by renal vasoconstriction, which offset the increase in filtered load, hypercalcemia also causes a decline in tubular reabsorption of calcium by both PTH dependent and -independent effects. Hypocalcemia decreases renal calcium excretion by decreasing the filtered load and enhancing the tubular reabsorption of calcium [32, 33]. Expansion of the extracellular fluid is associated with an increase in sodium, chloride, and calcium excretion, whereas reciprocal effects are seen with volume contraction. The mechanisms of these effects are interrelated with the effects of sodium reabsorption and compensatory changes that occur as a result of volume expansion [32]. Acute and chronic metabolic acidosis can be associated with an increase in calcium excretion, independent of PTH changes. The calciuria may in part be due to the mobilization of calcium from bone, as the hydrogen ion is buffered in the skeleton; however, direct effects of acidosis on tubular calcium resorption also play a role [32, 34]. Loop diuretics decrease calcium absorption as a result of inhibition of the transport of sodium chloride at the NKCC2 transporter in the TAL. Thiazide diuretics, which act in the DCT are associated with hypocalciuria [32, 34]. Two main mechanisms have been proposed to explain the effect of thiazides on calcium excretion [7]: (1) increased proximal sodium and water reabsorption due to volume depletion, and (2) increased distal calcium reabsorption at the thiazide-sensitive site in the DCT.

7. Calcium sensing receptor and partner proteins

The calcium-sensing receptor (CaSR) was primarily identified as the molecular sensor of free ionized calcium, enabling it function as the master regulator of systemic calcium metabolism in calciotropic tissues (parathyroid, thyroid C cells, kidneys, bone). The CaSR is ubiquitously expressed and as a result also expressed in non-calciotropic tissues like cardiovascular, nerve, pancreas, liver, lungs, prostate, GI, and breast tissues; implying a wide range of biological and cellular functions regulated by this receptor [35]. Due to the diversity and versatility of the CaSR-mediated signalling, the CaSR play varying roles in different tissues and pathologies.

7.1 CaSR gene and transcription

CaSR is a member of the C family of guanine protein-coupled receptors (GPCR) or seven-pass transmembrane domain receptors encoded by the CaSR gene composed of 8 exons (fig. 5B) and localized on the long arm of chromosome 3 (3q13.3-21). Exons1A and 1B encode alternative 5'-untranslated regions (UTRs) that splice to exon 2 encoding the AUG initiation codon (fig. 5B).

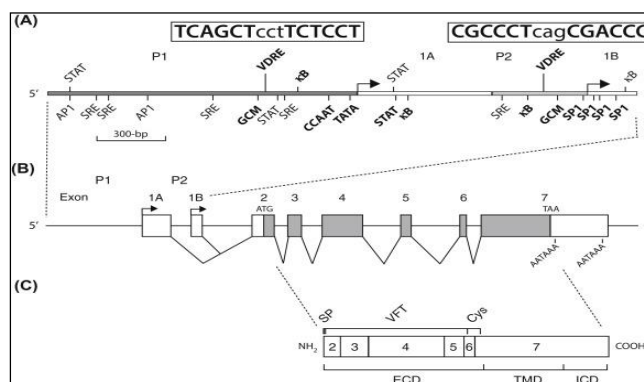


Figure 5 Calcium-sensing receptor: Gene, messenger RNA (mRNA), and protein

(A) CASR gene has two promoters, P1 and P2, gray bars, upstream of exons 1A and 1B, white bars, respectively. Arrows show transcription start sites. CCAAT and TATA boxes and SP-1 sites driving transcription of exon 1A and 1B, respectively, that are bolded. Cis-acting elements are shown. Vitamin D response element (VDRE) with complete 6-bp half-sites and 3-bp spacer is shown. Bolded: those shown to be functionally active. Not bolded: those predicted but either not functionally active or not yet evaluated. Not all predicted cis-acting elements are shown. (B) Exon/intron organization of the CASR gene. Exons are drawn to scale introns are not. White bars: mRNA untranslated (exons; 1A, 1B, part of 2, part of 7). Gray bars: mRNA protein coding (exons; part of 2, 3–6, part of exon 7). Alternative splicing of exons 1A and 1B to exon 2 is shown. (C) CaSR protein: 1078 amino acid (aa) protein encoded by exons 2–7. AATAAA, polyadenylation signals; AP1, activator protein 1; ATG, initiation codon; Cys, cysteine-rich domain; ECD, extracellular domain; GCM, glial cells missing; ICD, intracellular domain; SP, signal peptide; SRE, serum response element; STAT, signal transducer and activator of transcription; TAA, stop codon; TMD, transmembrane domain; VFT, Venus flytrap domain; κB, kappa-B element responsive to nuclear factor kappa-light-chain-enhancer of activated B cells.

Promoter P1 has TATA and CCAAT boxes upstream the transcription start site of exon 1A and promoter P2 has Sp1/3 motifs at the start site of exon 1B (fig. 5A). Exons 2–7 encodes 1078 amino acid CaSR protein and divided into 5 regions (fig. 5C): signal peptide, ECD subdivided into venus flytrap and cysteine rich domains, TMD, and ICD. The ECD is the amino terminal containing the VFT structure where majority of ligand binding sites of the CaSR are located. The TMD is composed of 7 transmembrane helices, where other ligand binding sites are found [36]. A cysteine-rich domain exists between the ECD and the first transmembrane helix of TMD. The ICD is the carboxyl terminal tail that contains protein kinase C, A and D phosphorylation sites [36].

Human CaSR transcription is driven by either promoter P1 with a TATA and CCAAT box or P2 with an Sp1/3 site at the transcriptional start site. Both promoters drive significant levels of basal activity, with promoter P2 being 2.5-fold more active than P1 in most cell types. However, pathophysiological differences exist in the type of CaSR gene promoter engaged. Greater exon1B transcripts relative to exon1A transcripts occur in human thyroid C-cells and renal PT cells. Some of the factors and mechanisms involved in transactivation of the CaSR gene have been identified. Transcription factors bind their specific sites to regulate the transcription of CaSR gene: active vitamin D binds VDRE, NF-κB/TNF-α and IL-1β binds kappa B elements, IL-6 binds STAT1/3 and Sp1/3 elements, GCM2 binds GCM response elements [37]. The CaSR gene is highly expressed in the parathyroid gland and renal tubule. However, ubiquitously expressed at lower levels in several other tissues: liver, bone, lung, breast, placenta, vasculature and gut [37]. Regulated CaSR gene expression is important in growth and development, both in normal adult physiology, and disease pathogenesis.

7.2 CaSR Ligands

The CaSR is a “promiscuous” receptor that recognizes and binds several ligands (table 2). Extracellular Ca²⁺ is the CaSR's most physiologically relevant agonist in vivo. However, other physiological ligands also modulate the activity of CaSR (table 2).

Table 2 CaSR ligands and physiological conditions which affect CaSR activity

True agonists or Orthosteric agonist	
Cations	
High affinity	Gd ³⁺ , La ³⁺ , Eu ³⁺ , Tb ³⁺
Medium affinity	Zn ²⁺ , Ni ²⁺ , Cd ²⁺ , Pb ²⁺ , Cu ²⁺ , Fe ²⁺

Low affinity	Ca ²⁺ , Mg ²⁺ , Ba ²⁺ , Mn ²⁺ , Sr ²⁺
Polyamines	
Spermine, spermidine, putrescine	
Aminoglycoside antibiotics	
Neomycin, paromomycin, tobramycin, gentamicin, kanamycin	
Positive Allosteric Modulators	
Endogenous: large aromatic l-amino acids: Phe, Trp, Tyr, His	
Basic polypeptides: poly-l-arginine, poly-lysine, protamine, amyloid β peptide	
Calcimimetics: Cinacalcet, NPS R467, NPS R568, AMG641	
Negative Allosteric Modulators	
Calcilytics: NPS 2143, Calhex 231, Ronacaleret	
Physiological state	
Ionic strength and body pH	

CaSR ligands are classified into true agonists, and allosteric modulators. True agonists directly activate the CaSR independent of any other ligand, while allosteric modulators require the binding of an orthosteric agonist in millimolar concentrations to the CaSR to elicit its effect [38]. An orthosteric antagonist and endogenous negative allosteric modulator of the CaSR is yet to be identified [38]. Extracellular Ca²⁺ is the main orthosteric physiological agonist of the CaSR. Five putative Ca²⁺ binding sites are present in the ECD of the CaSR. By definition, the receptor binding sites for allosteric modulators are topographically distinct from that of the true agonists. For example large aromatic l-amino acids, bind to receptor sites different from Ca²⁺ and other cations to act as positive allosteric modulators of CaSR activation [35]. Ligand binding to the CaSR modulates subsequent ligand-receptor interaction, leading either to homotropic cooperativity if the ligands are identical, or to heterotropic cooperativity, if they are different (i.e. true agonist and allosteric modulator) [35]. In heterotropic cooperativity, the binding of an allosteric ligand alters the receptor conformation such that the binding affinity and/or the signaling capacity of the orthosteric ligand is changed. Heterotropic cooperativity may therefore be in a positive or a negative direction and as such, allosteric ligands are categorized as positive (agonist) or negative (antagonist) allosteric modulators [39]. Allosteric modulators like calcimimetics (Cinacalcet-Sensipar, evocalcet, etelcalcetide, velcalcetide) and calcilytics does not only modulate the activity of the CaSR, but also regulates the expression of CaSR. Calcimimetics upregulates the expression of CaSR, while calcilytics either downregulate or upregulate CaSR expression depending on the cell type [35]. As allosteric agonists of extracellular Ca²⁺ at CaSR, calcimimetics mimics the action of calcium on cells and as a result exerts no effect in the absence of extracellular Ca²⁺. Physiologic conditions such as ionic strength and pH also regulates CaSR activity. Ionic strength inactivates, while high pH levels (>7–7.8) activate the CaSR [35].

7.3 CaSR signalling

The activation of CaSR by several ligands induces unique conformations of the CaSR leading to preferential coupling to different G-proteins, a phenomenon referred as ligand-bias signaling or biased agonism [40]. The spectrum of CaSR ligands with their respective G protein activation and downstream signalling pathways is presented in figure 6. CaSR signalling depends on the type of cell, expression of G protein isoforms, their binding affinity and rate of deactivation of the receptor, type of ligands, intracellular enzymes content, and adapter proteins that control the assembly of signalling scaffolds, and mutations of the receptor [35].

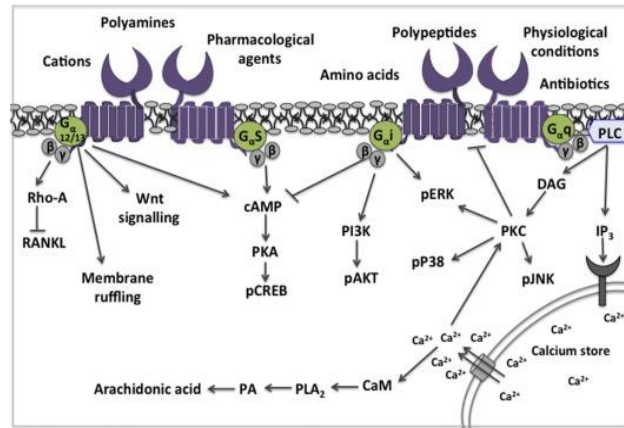


Figure 6 G protein activation and downstream signalling of the active CaSR

When Ca^{2+} binds to the CaSR, it undergoes a conformational change within the ECD, which are transmitted through the TMD to induce the coupling of ICDs with guanine nucleotide (G) protein subunits. Four classes of guanine nucleotide- α ($G\alpha$) subunits (G_i/o , G_s , $G_q/11$ and $G_{12/13}$) engage the CaSR in mediating various downstream signaling pathways (figure 6) [41]. Intracellular Ca^{2+} signal, cAMP synthesis, and protein phosphorylation are some of the key signalling events regulated by the CaSR (figure 6). Specifically, the CaSR-G protein interaction regulates the concentration or activity of several intracellular mediators/messengers including phospholipases, inositol 1, 4, 5-trisphosphate (IP₃), diacylglycerol (DAG), protein kinases, cytoplasmic free Ca^{2+} and cyclic adenosine monophosphate (cAMP), within tissue cells (Figure 6). The adaptor-protein-2 sigma ($\text{AP}2\sigma$) subunit encoded by *AP2S1* gene engages the CaSR to cause receptor internalization by endocytosis.

When activated CaSR interacts with $G_{q/11}$, it leads to phospholipase C (PLC) activation which hydrolysis phosphatidylinositol-4, 5-bisphosphate [Ptdins(4,5)P₂] via inositol polyphosphate-5-phosphatase (OCRL1 protein) to form IP₃ and DAG (figure 6). IP₃ stimulates intracellular Ca^{2+} (iCa^{2+}) release from stores while DAG mediates the activation of protein kinase C (PKC) that further activates phospholipase (PL) A, C, and D (Figure 6). Activation of PLA₂ enhances the synthesis of arachidonic acid and activation of phosphatidylinositol 4-kinase which catalysis the replenishment of Ptdins(4,5)P₂ [42]. The CaSR itself can be phosphorylated by PKC on its PKC-phosphorylation sites. CaSR-dependent PLC-IP₃- iCa^{2+} signal pathway has been observed in liver cells, parathyroid cells, lung epithelial cells, and kidney cells [35]. Inactivating mutations of oculocerebrorenal syndrome of Lowe (OCRL) gene in calciotropic tissues leads to blunting of CaSR-mediated IP₃ production resulting in calcium and phosphate disorders [43].

The coupling of CaSR to $G_{i/o}$, leads to inhibition of adenylate cyclase (AC) decreasing the concentration of 3',5'-cyclic adenosine monophosphate (cAMP), whereas upregulation of AC activity occurs via the interaction of CaSR with the G_s subunit, increasing intracellular levels of cAMP in tissues. Upregulation of cAMP activates PKA signaling, Ca^{2+} /calmodulin (CaM)/calcineurin, mitogen activated protein kinases (MAPKs), and extracellular signal-regulated kinases (ERKs) pathways [35]. CaSR-cAMP mediated signaling pathways further engages several complex downstream signaling pathways that enhance cellular processes including cell secretion, proliferation, differentiation, chemotaxis, apoptosis, gene expression, and ion channel switch [44]. $G_{i/o}$ -mediated CaSR suppression of cAMP levels has been reported in medullary TAL cells, and in normal breast cells. Whereas G_s -mediated CaSR upregulation of cAMP levels is observed in pituitary, ovary, and cancer cells (breast, colon) [35]. In the parathyroids and kidneys, CaSR-mediated cAMP signaling respectively controls PTH secretion and Ca^{2+} transport in renal tubules. Another key intracellular pathway mediated by CaSR- $G_{i/o}$ interaction and involved in cellular processes is the phosphatidylinositol 3 kinase-PI3K (PI3K-PKB/AKT) pathway. CaSR via PKC activates janus kinases (JKNs), Phospholipases A, C, and D to promote cellular processes [44]. $G_{12/13}$ -mediated CaSR interaction with filamin A (an inhibitor of CaSR degradation), activates Rho GTPases (Rho A) pathways that regulates actin stress fiber formation and actinomycin contraction involved in cytoskeletal maintenance. $G_{12/13}$ -mediated CaSR signaling modulates other cell architecture maintenance pathways such as increase in E-cadherin levels and subsequent β -catenin release. β -catenin is an important proto-oncogene, which is a member of the Wnt pathway family that enhances cell-cell and cell-matrix adhesion via the actin-based cytoskeleton [44]. $G_{12/13}$ -mediated CaSR signalling also regulates the production of cAMP [35].

Activated CaSR regulates the expression of various transcription factors which engage complex signal pathways in mediating cellular processes [44]. The activation of CaSR could impair nuclear translocation of β -catenin, leading to decreased β -catenin-TCF4 complex formation and down regulation of cyclin D family genes, c-Myc, and c-Fos. This

inhibits cell proliferation and Wnt pathway [44]. The CaSR mediates NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), IL-1 β , and TNF α signaling via κ B response element on CaSR gene. NF- κ B is responsible for the expression of immune response proteins, inflammatory factors, and other several intracellular proteins involved in physiologic and pathologic processes [37]. CaSR via G12/13 mediates the activation of Wnt5a and Rho A which suppress NF κ B and RANKL. RANKL affects inflammation and tissue growth, it particularly regulates bone regeneration and remodeling.

7.4 CaSR and calcitropic tissues

A need for a tight regulation of extracellular Ca^{2+} necessitates the evolution of a complex homeostatic system to maintain Ca^{2+} at near-constant concentrations (Figure 7). The CaSR expressed in parathyroid glands, thyroid C cells, kidneys and bones closely monitor blood calcium level by engaging downstream signaling pathways presented in figure 6 [45]. CaSR mediated maintenance of systemic calcium homeostasis, occur by maintenance of balance between GI absorption of Ca^{2+} , renal excretion of Ca^{2+} , and the release of Ca^{2+} from the bone (figure 7).

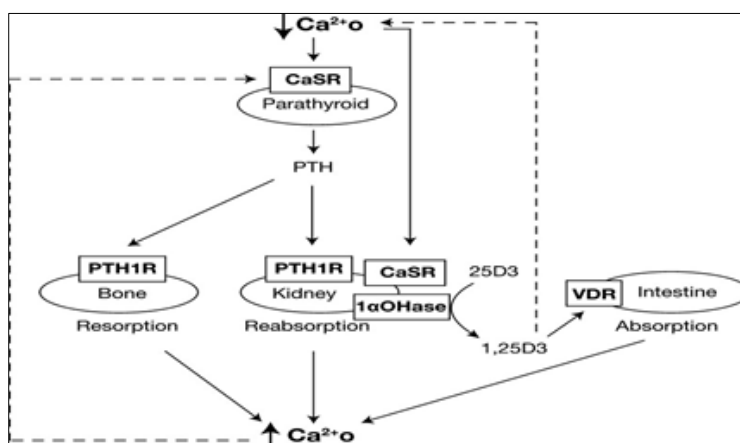


Figure 7 CaSR coordination of calcium regulation

The CaSR in the parathyroid senses minute changes (increases or decreases) in serum calcium levels (1.1–1.3 mmol) and generates downstream signaling pathways that induce cellular processes involved in PTH secretion and synthesis. Regulators of CaSR expression/activity and PTH release/synthesis include; extracellular Ca^{2+} , phosphate, vitamin D, proinflammatory cytokines, certain drugs, transcription factors. Low plasma calcium levels, low vitamin D levels and high phosphate levels stimulate the synthesis and release of PTH, by reducing the expression and activity of CaSR (figure 8). Rapid PTH release from secretory granules in the parathyroids during hypocalcemia is modulated by the binding of extracellular Ca^{2+} to CaSRs on parathyroid chief cells (Figure 8). Conversely, high plasma levels of calcium and vitamin D upregulates the expression and activity of CaSR, while PTH synthesis and release is reduced [37].

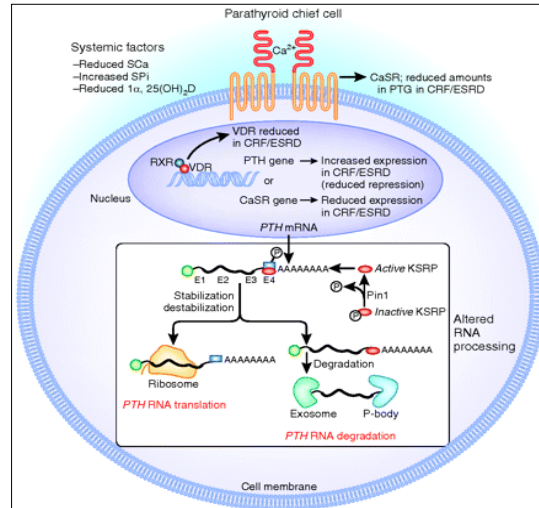


Figure 8 Regulation of CaSR and PTH expression

Proinflammatory cytokines (TNF- α and IL-1 β , and IL-6) upregulates the expression of CaSR gene via NF- κ B in parathyroid, thyroid C, bone and kidney cells to provoke altered systematic Ca²⁺ homeostasis. Proinflammatory cytokines signals the release of NF- κ B from cytoplasmic I κ B, NF- κ B translocates to the nucleus and binds specific κ B response elements on the promoter regions of CaSR to up regulate CaSR gene transcription in calciotropic and non-calciotropic tissues [37]. Hypocalcemia, which is common in inflammatory conditions like rheumatoid arthritis, critically ill patients (sepsis, major burns) and non-acutely ill patients undergoing surgery are effects of increased expression of CaSR in calciotropic tissues [37].

The CaSR and PTH gene could be transactivated by the glial cells missing-2 (GCM2) protein encoded by GCM2 gene. GCM2 binds on GCM response elements on both PTH/CaSR gene to regulate their expression. v-maf musculoaponeurotic fibrosarcoma oncogene homolog B (MafB) is a transcription activator, expressed in parathyroid glands and associates with GCM2 to synergistically activate PTH gene expression [46]. The transcription factor, GATA3 cooperates with GCM2 and MafB to activate PTH gene expression by interacting with the ubiquitous specificity protein-1 (SP1) transcription factor [47]. Reduced expression of parathyroid GCM2 correlates with decreased expression of the CaSR. Hyperactive forms of GCM2 may subsequently contribute to parathyroid hyperactivity or tumorigenesis. In addition to transactivation of PTH gene, increased expression of GCM2 in the parathyroid glands maintains parathyroid cell differentiation and development [48].

Active vitamin D transactivates PTH and CaSR gene. Active vitamin D inhibits PTH expression and upregulates the expression of CaSR. Active vitamin D (1,25(OH)₂D) regulates the synthesis and release of PTH by directly regulating the rate of transcription of PTH gene. 1,25(OH)₂D reduces the rate of transcription of the PTH gene leading to low plasma concentration of PTH. 1,25(OH)₂D binds to the vitamin D receptor (VDR) in the nucleus of the chief cells of parathyroid gland and further associates with the retinoic acid X receptor (RXR) to form a complex which binds to vitamin D response element (VDRE) within the promoter region of the PTH gene [37]. Reduced expression of CaSR, hyperphosphatemia, hypocalcemia, and low levels of 1,25(OH)₂D induces reduced expression of VDR. Active vitamin D deficiency, including its reduced concentrations in chronic kidney disease (CKD) or end stage renal disease (ESRD) and resistance to vitamin D due to reduced expression of VDR leads to increased PTH expression (or reduced repression) and hyperparathyroidism [37]. Active vitamin D induces CaSR upregulation in the chief cells to improve the responsiveness of the parathyroid gland to extracellular Ca²⁺ resulting to decrease in PTH secretion. The inhibitory action of extracellular Ca²⁺ on PTH synthesis, secretion and parathyroid cell proliferation could be ameliorated by supplementation with active vitamin D. Other than the parathyroid gland, the expression of CaSR is also altered by 1,25(OH)₂D in the kidney, and in thyroid C cells, such that 1,25(OH)₂D via VDR-VDRE upregulates the expression of CaSR in thyroid C cells and kidney [37].

Besides transcriptional regulation of PTH gene expression via CaSR signaling, post-transcriptional mechanisms also mediates PTH expression by the interaction of RNA-binding proteins as well as micro RNAs (miRNAs) with PTH mRNA on specific elements of its 3'untranslated regions (3'UTRs). After export from the nucleus, PTH mRNA transcripts may interact with two adenosine uridine-rich elements (ARE)-binding proteins (ARE-BPs): AU-rich binding factor (AUF1) and K-homology splicing regulatory protein (KSRP) via AREs (figure 8). AUF1 encoded by HNRNPD gene and KSRP

regulates PTH mRNA half-life and stability within the chief cells. It is important to note that long-term replenishment of PTH stores to ensure availability during needs is dependent on the stability of PTH mRNA and its subsequent translation into prepro-PTH [49]. The fate of PTH mRNA is partly dependent on the relative concentration of stabilizing and destabilizing ARE-binding proteins. AU-rich binding factor (AUF1) stabilizes PTH mRNA, resulting to increases in PTH mRNA half-life and its subsequent translation into prepro-PTH. Activated KSRP destabilizes PTH mRNA culminating into its decay or degradation while inactivated KSRP stabilizes PTH mRNA and increases its half-life. miRNA expression regulates PTH gene expression by destabilization of PTH mRNA resulting in PTH mRNA degradation and/or translation repression [50]. Stabilized PTH mRNA undergoes translation in ribosomes, whereas destabilized mRNA are degraded in exosomes. The activities of AUF1 and KSRP are regulated by changes in extracellular Ca^{2+} and phosphate. Peptidyl-prolyl cis-trans isomerase NIMA-interacting-1 (Pin1), a peptidyl-prolyl isomerase, alters KSRP phosphorylation and the binding of KSRP to the AREs PTH mRNA [50]. Peptidyl-prolyl cis-trans isomerase NIMA-interacting-1 (Pin1) dephosphorylates inactive KSRP to activated KSRP (figure 8). Reduced activity or expression of Pin1 enhances the phosphorylation of KSRP to its inactive state, inactive KSRP promotes the stability of PTH mRNA with concomitant increase in PTH production (figure 8). Inactivation of Pin1 reduces the ratio of the ARE-BPs (active KSRP: AUF1) in favor of AUF1 induced stabilization of PTH mRNA and PTH synthesis. Low serum Ca^{2+} and high phosphate levels reduce the activity of Pin1, which induces hyperparathyroidism via inactive KSRP [51]. Regulations in the binding or activity of Pin1 and KSRP are tightly controlled by several post-translational mechanisms some of which are probably mediated by CaSR signaling pathways [50]. Protein kinase A (PKA) and p38 MAPK pathways respectively mediates the phosphorylation of Pin1 and KSRP to impair interactions with their target molecules [50]. microRNAs binds PTH genes/mRNAs, as well as CaSR gene and epigenetically regulate their expression in normal and tumorigenic parathyroid cells [52].

Overall, genetic and epigenetic mechanisms alter PTH and CaSR expression. The consequence of reduced expression and inactivation or loss in function of CaSR in the parathyroid is hypercalcemia or severe hyperparathyroidism. Whereas, increased expression and activation or gain in function of CaSR is hypocalcemia and hypoparathyroidism [35].

CaSR is expressed in the various segments of the renal tubule (table 3) and regulates ion transport, renin secretion, urinary acidification and concentration [26].

Table 3 Intrarenal distribution, targets, and effectors of the CaSR

Region	CaSR action	Biological Effects
PCT/PST	↓PTH1R expression ↑1-Hydroxylase activity ↑p38 MAPK activity	↓Phosphate transport ↑1,25(OH) ₂ D synthesis ↑VDR expression
MTAL	↑H ⁺ -K ⁺ -ATPase activity ↓Calcitonin- and AVP-induced cAMP production	↑Urine acidification ↓NaCl/Ca ²⁺ /Mg ²⁺ transport
CTAL	↓CLDN-16 ↓NKCC2 ↓ROMK ↓PTH-induced second messenger production	↓Ca ²⁺ /Mg ²⁺ transport ↓NaCl/ Ca ²⁺ /Mg ²⁺ transport ↓NaCl/ Ca ²⁺ /Mg ²⁺ transport ↓Transcellular Ca ²⁺ transport
DCT/CNT	↑TRPV5	↑Ca ²⁺ reabsorption
CCD/OMCD	↑H ⁺ -ATPase, H ⁺ secretion	↑Urine acidification, ↑urine Ca ²⁺ removal to prevent renal stone formation
OMCD/IMCD	↓AVP-dependent AQP2 apical expression, water reabsorption	↓Urine concentration, ↑urine Ca ²⁺ removal to prevent renal stone formation
JG cells	↓AC-V activity, renin gene expression	↓Renin secretion

CaSR, calcium-sensing receptor; PCT/PST, proximal convoluted/straight tubule; MTAL, medullary thick ascending limb (TAL); CTAL, cortical TAL; DCT/CNT, distal convoluted tubule/connecting segment; CCD, cortical collecting duct; OMCD/IMCD, outer/inner medullary collecting duct; JG, juxtaglomerular; PTH, parathyroid hormone; MAPK, mitogen-activated protein kinase; NKCC2, Na⁺-K⁺-2Cl⁻ cotransporter 2; ROMK, renal outer

medullary potassium K⁺ channel; TRPV5, transient receptor potential vanilloid 5; AQP2, aquaporin 2; AC-V, type V adenylate cyclase; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; AVP, arginine vasopressin

Consequently, reduced expression, inactivation or loss in function of CaSR stimulates the renal reabsorption of calcium leading to hypocalciuria. Increased expression, activation or gain in function of CaSR in renal cells usually independent of PTH, directly inhibits the paracellular uptake of calcium/cations in the cortical thick ascending limb of the distal nephron leading to hypercalciuria [53]. Inactivating mutations of CaSR associated with decreased transcriptional activity of CaSR gene promoter 1 and its renal tubular expression predisposes to hypercalciuria and nephrolithiasis [54]

The CaSR expressed in bone cells modulates bone cell metabolism and remodeling (osteogenesis and resorption) which in turn regulates calcium homeostasis. Loss of function or inactivation of the CaSR in bone cells has been shown to influence bone turnover [55]. In old age, it is shown that activation of CaSR enhances osteoblast differentiation, survival, proliferation and matrix production [56]. Activation of CaSR in osteoclasts inhibits bone resorption in young age, and conversely promotes bone resorption in older subjects. The interaction of activation of CaSR and PTH leads to net bone formation in trabecular bone or net bone formation in cortical bone [55]. Unlike a reduced PTH secretion by the parathyroid during activation of CaSR in hypercalcemic states, activation of the C cells CaSR in the thyroid gland conversely releases more calcitonin into circulation. In hypocalcemic states, inactivated CaSR mediates the reduction of calcitonin secretion by thyroid C cells. Reduction in calcitonin levels enhances body replenishment of calcium, since calcitonin inhibits bone resorption and increases urinary Ca²⁺ excretion [35].

7.5 CaSR and non-calcitropic tissues

Besides its primary function as a regulator of calcium homeostasis, the CaSR play roles in several physiologic/pathologic processes, and shows promise as therapeutic targets in a variety of disease treatment [35]. The CaSR is present in the smooth muscles of blood vessels, heart, lungs, intestine and play roles in the normal functioning of these tissues. In the cardiovascular system, the CaSR regulates blood vessel tone and blood pressure [28]. In the central nervous system, spermine and spermidine secreted along with other neurotransmitters activates the CaSR. Spermine and spermidine activation of CaSR promotes transient increases in cytoplasmic Ca²⁺ by mobilization of intracellular calcium stores via the activation of PLC [57]. Thus CaSR regulates neural cell growth, development, and function. CaSR is present in adipocytes and hepatocytes. In the epidermal tissue, the CaSR regulates intracellular Ca²⁺ signalling and E-cadherin to enhance cell-cell (keratinocytes) adhesion, differentiation and survival [58]. In breast tissue, CaSR is involved in lactation and enables Ca²⁺ transport into milk by modulating the production of PTHrP [59].

The CaSR is expressed in tissues of the digestive system: pancreas, liver, and the GIT. In the pancreas, the CaSR is highly expressed in the endocrine islets, pancreatic ducts, exocrine acinar cells, intra-pancreatic nerves and the blood vessels. The CaSR mediates intracellular Ca²⁺ signalling and cAMP-dependent pathways to control exocrine (juice, enzymes, bicarbonate, Ca²⁺) and endocrine (insulin) secretions of the pancreas. Activation of the CaSR stimulates ductal bicarbonate secretion and decrease Ca²⁺ concentration in pancreatic juice. CaSR regulates beta-cell proliferation, cell-cell communication and cellular adhesion (via E-cadherin) which promotes insulin secretion [44]. In the liver, activated CaSR stimulates bile flow via PLC-IP₃ signal transduction pathway [44]. The CaSR is expressed along the entire GI tract including the taste buds which participates in taste regulation, oesophagus, stomach (gastric epithelial glands and ganglions), and the colon. In the esophagus, activated CaSR increases intracellular Ca²⁺ levels and activates ERK1/2 (MAPK) signal pathways to promote the multifunctional cytokine IL-8 (CX-CL8) secretion [44]. In the gut the CaSR serve as a nutrient sensor by binding ligands present in nutrients to enhance enterocyte absorptions and secretions [44]. In the stomach, the CaSR enhances the elevation of intracellular Ca²⁺ to stimulate parietal cell H⁺-K⁺-ATPase activity which subsequently induces gastrin, calcitonin and gastric acid secretion by the stomach cells [44]. The PLC-IP₃ and ERK1/2 (MAPK) as well as Ca²⁺-dependent and Ca²⁺-independent protein kinase C (PKC) isoforms are the key signalling pathways involved in CaSR mediated acid secretion by stomach cells [44]. The CaSR is involved in regulating intestinal nutrient absorption and fluid-water-ion transport (secretion and absorption) to maintain water and electrolyte homeostasis. Bicarbonate (HCO₃⁻) secretion in the colon is modulated by CaSR-cAMP pathway. Peptides/amino acids present as digestive products in the intestines activates the CaSR which regulates the secretion of cholecystokinin (pancreozymin) and Ghrelin from enteroendocrine cells [44]. Cholecystokinin is a peptide hormone that enhances effective digestion, which also serve as a hunger (or appetite) suppressant. Ghrelin is a potent orexigenic hormone that stimulates hunger via the CNS and regulates glucose metabolism by the inhibition of insulin secretion [54]. CaSR modulates colonic myofibroblasts secretion of bone morphogenetic protein 2 and Wnt5a involved in the regulation of normal intestinal epithelial cell proliferation and differentiation.

Changes in tissue CaSR expression mediate tumorigenesis, several inflammatory diseases, secretory and metabolic disorders. Alterations in CaSR expression and its intracellular pathways (NF-KB, cAMP, MAPK/ERKs) lead to changes in

cell proliferation, differentiation, migration, adhesion, secretion, apoptosis, and angiogenesis. Alterations in CaSR expression occur in benign and malignant tumors implying that CaSR could either be a tumor suppressor or oncogene depending on the cell type. As a tumor suppressor, epigenetic changes involving hypermethylation of the GC rich P2 promoter, histone acetylation, and microRNA post-transcriptional silencing of CaSR mRNA are responsible for reduced expression or complete absence of CaSR in neuroblastomas, colorectal tumors and parathyroid adenomas. In parathyroid adenomas, the expression of exon1A transcripts driven by the upstream promoter (P1) is decreased; whereas expression of exon1B transcripts driven by the usually stronger promoter P2 is unchanged resulting in decreased CaSR mRNA and protein expression. Altered expression of miRNAs may be involved in the development of parathyroid tumors [37]. CaSR expression levels is increased in breast, renal, gastric, ovarian, prostate, and testicular cancers in which the CaSR acts as an oncogene [59]. However, specific molecular mechanisms underlying enhanced expression of CaSR in these oncogenic tumors are yet to be identified. Humoral hypercalcaemia of malignancy is a paraneoplastic syndrome often linked with oncogenic tumors associated with increased expression of CaSR that

metastasize to the bone. These tumors synthesize PTHrP which binds PTHR1 on bone to stimulate osteolytic bone destruction leading to hypercalcemia [35].

Asthmatic patients express high levels of CaSR in their respiratory airways as a result of receptor stimulation by ligands including calcium and allergens which increases airway hyperreactivity and inflammation [60]. Liver injury releases substances which activate CaSR mediated inflammatory signaling; by enhancing phosphorylations in the p38 MAPK and ERK-1/2 signal pathway to induce hepatitis and hepatocyte apoptosis. Acute and chronic pancreatitis has been shown to be associated with activating mutations of CaSR receptor [44]. In oesophagitis, activated CaSR induces the proliferation of esophageal epithelial cells and as a result, implicated in the proliferative response to injury and pathogenesis of oesophagitis [44].

Metabolic syndrome is associated with hereditary CaSR disorders in regards to its involvement in the regulation of insulin secretion, post prandial glucose regulation, lipolysis, and inhibition of myocardial cell proliferation. Hypercalcemic patients present with gastric hyperacidity and hypergastrinemia due to stomach cell CaSR activation. Vascular calcification the hallmark of atherosclerosis and chronic kidney disease during aging and pathologies is inversely related with the expression of the CaSR [37].

7.6 CaSR and therapeutics

The regulation of CaSR activation and expression in both calcitropic and non calcitropic tissues by certain drugs could serve therapeutic purposes in the management of disorders linked with CaSR. Although both positive (calcimimetics) and negative (calcilytics) allosteric modulators of the CaSR are already in development, currently only cinacalcet, is approved for use in humans.

In the parathyroids calcimimetics suppresses PTH secretion and as a result therapeutically indicated in hyperparathyroidism and related hypercalcemia. Calcimimetics transiently increase calcitonin secretion from thyroidal C cells [35]. Cinacalcet is indicated in patients on dialysis by reducing the levels of calcium, phosphate, FGF23, and parathyroids hyperplasia. Cinacalcet stimulates bone remodeling, with bone gain resulting in diminished skeletal fracture rates and reduced need for parathyroidectomy in chronic kidney disease associated secondary hyperparathyroidism. It is also indicated in primary hyperparathyroidism, parathyroid carcinomatosis, parathyromatosis and inoperable parathyroid surgery. In parathyroid cancer, cinacalcet reduces hypercalcaemic symptoms, without ameliorating parathyroid cancer progression. However, cinacalcet shows promise in the amelioration of malignancy resulting in good prognosis in colorectal, neuroblastoma, and parathyroid cancers by impairment of cell proliferation, cell growth, and cell-cell adhesion [59]. Evidence shows that the CaSR is a central mediator of the anti-tumourigenic effects of calcium and as a result, increased intake of calcium reduces the risk of several cancers. Also activated CaSR enhances the sensitivity of human colon carcinoma cells to chemotherapeutic drugs (mitomycin C and fluorouracil) [61]. Activation of CaSR ameliorates portal hypertension and serve as a therapeutic target for the prevention and treatment of drug- or alcohol-induced liver disease. Increasing CaSR expression and decreasing mineralization of the vascular smooth muscle cells with active vitamin D or cinacalcet show promise in the treatment of vascular calcification and arterial hypertension [37]. CaSR has anti-inflammatory, anti-secretory, pro-absorbent and inhibitory properties on intestinal motility. Upregulation of CaSR expression has been shown to induce negative feedback inhibition of synthesis of TNF α [62]. Thus activation of CaSR with cinacalcet is a potential therapeutic approach for diarrhea and intestinal inflammation [54]. Common side effects of calcimimetics which include: nausea and vomiting, hypocalcemia and adynamic bone disease especially when intact parathyroid hormone (iPTH) levels drop below 100pg/mL, limits its use.

Calcilytics, an allosteric antagonist of extracellular Ca^{2+} at CaSR induces a transient increase in PTH secretion, which temporarily exerts anabolic effects on bone tissue, resulting into increases in bone volume and density due to increased bone deposition and resorption. Calcilytics, shows promise in the reduction of cell proliferation, cell-cell adhesion and cell growth to achieve good prognosis in malignancies mediated by CaSR activation [59]. Therapeutic applications of calcilytic drugs also extend to neurological disorders and asthma [63]. However, prolonged use of calcilytics decreases PTH production causing bone resorption, and degradation of bone resulting hypercalcemia. Inactivation of respiratory airway CaSR with calcilytics by nebulization has been shown to elicit anti-inflammatory and anti-allergic properties, in the therapeutic management of asthma [60]. Antagonizing CaSR signaling in human astrocytes and neurons with calcilytics is key to halting Alzheimer's disease progression [64].

8. Conclusion

Calcium is an essential ion required in the body for many vital intracellular and extracellular functions including skeletal support. The regulation of calcium homeostasis is largely mediated by PTH, calcitriol and the CaSR which tightly regulates ion transport by the kidneys, and intestinal tract. This review provides the clinician with basic information necessary for the diagnosis and therapeutic management of disorders related to regulatory systems of calcium metabolism.

Compliance with ethical standards

Acknowledgments

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References

- [1] Goldstein DA. Serum Calcium. In: Walker HK, Hall WD, Hurst JW, eds. *Clinical Methods: The History, Physical, and Laboratory Examinations*. 3rd ed. Boston: Butterworths. 1990. p. 677-679.
- [2] Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC, Taylor CL, Yaktine AL, et al., eds. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US). 2011. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK56070/> doi: 10.17226/13050
- [3] Guyton AC. Parathyroid Hormone, Calcitonin, Calcium and phosphate metabolism, Vitamin D, Bone and Teeth. *Guyton and Hall Text Book of Medical Physiology*. 12th ed. Philadelphia: Saunders Elsevier; 2001. p. 899-915.
- [4] Rosol TJ, Chew DJ, Nagode LA, Capen CC. Pathophysiology of calcium metabolism. *Veterinary Clinical Pathology*. 1995; 24(2): 49-63.
- [5] Rasmussen H. The cycling of calcium as an intracellular messenger. *Scientific American*. 1989;261(4):66-73.
- [6] Brown EM, Pollak M, Seidman CE, Seidman JG, Chou YH, Riccardi D, Hebert SC. Calcium-ion-sensing cell-surface receptors. *The New England Journal of Medicine*. 1995;333(4):234-40.
- [7] Blaine J, Chonchol M, Levi M. Renal control of calcium, phosphate, and magnesium homeostasis. *Clinical journal of the American Society of Nephrology*. 2015;10(7):1257–1272.
- [8] Johnson JA, Kumar R. Renal and intestinal calcium transport: roles of vitamin D and vitamin D-dependent calcium binding proteins. *Seminars in Nephrology*. 1994;14(2):119-28.
- [9] Shaker JL, Deftos L. Calcium and Phosphate Homeostasis. [Updated 2018 Jan 19]. In: Feingold KR, Anawalt B, Boyce A, et al., eds. *Endotext* [Internet]. South Dartmouth (MA): MDTText.com, Inc. 2000. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK279023/>
- [10] Rizzoli R, Bonjour JP. Physiology of calcium and phosphate homeostasis. In: Seibel MJ, Robins SP, Bilezikian JP eds. *Dynamics of Bone and Cartilage Metabolism*. 2nd ed. San Diego: Academic Press; 2006. p. 345-360.
- [11] FAO/WHO. Vitamin and mineral requirements in human nutrition: Calcium. Report of a joint FAO/WHO expert consultation. Bangkok, Thailand: 2001. p. 154-156.
- [12] Perez AV, Picotto G, Carpentieri AR, Rivoira MA, Peralta Lopez ME, Tolosa de Talamoni NG. Minireview on regulation of intestinal calcium absorption. Emphasis on molecular mechanisms of transcellular pathway. *Digestion*. 2008; 77: 22-34.
- [13] Gumbiner BM. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell*. 1996; 84: 345-357.
- [14] Guillemot L, Paschoud S, Pulimeno P, Foglia A, Citi S. The cytoplasmic plaque of tight junctions: a scaffolding and signalling center. *BiochimBiophysActa* 2008; 1778: 601-613.
- [15] Hwang I, Yang H, Kang HS, Ahn C, Hong EJ, An BS, Jeung EB. Alteration of tight junction gene expression by calcium- and vitamin D-deficient diet in the duodenum of calbindin-null mice. *International Journal of Molecular Science*. 2013;14:22997-23010.
- [16] Karbach U. Paracellular calcium transport across the small intestine. *Journal of Nutrition* 1992; 122(3): 672-677.

- [17] Areco VA, Kohan R, Talamoni G, Tolosa de Talamoni NG, Peralta López ME. Intestinal Ca²⁺ absorption revisited: A molecular and clinical approach. *World Journal of Gastroenterology*. 2020; 26(24): 3344-3364.
- [18] Prentice A. Maternal calcium metabolism and bone mineral status. *American Journal of Clinical Nutrition*. 2000; 71 Suppl 5: 1312S-1316S.
- [19] Bullamore JR, Wilkinson R, Gallagher JC, Nordin BE, Marshall DH. Effect of age on calcium absorption. *Lancet*. 1970;2:535-537.
- [20] Ramsubeik K, Keuler NS, Davis LA, Hansen KE. Factors associated with calcium absorption in postmenopausal women: a post hoc analysis of dual-isotope studies. *Journal of the Academy of Nutrition & Dietetics*. 2014;114:761-767.
- [21] Gallagher JC. Vitamin D and aging. *Endocrinology & Metabolic Clinics of North America*. 2013;42:319-332.
- [22] Tolosa de Talamoni N, Marchionatti A, Baudino V, Alisio A. Glutathione plays a role in the chick intestinal calcium absorption. *Comparative Biochemistry & Physiology. Part A Physiology*. 1996;115:127-132.
- [23] Clark A, Mach N. The Crosstalk between the Gut Microbiota and Mitochondria during Exercise. *Frontiers in Physiology*. 2017;8:319.
- [24] Charoenphandhu N, Wongdee K, Krishnamra N. Is prolactin the cardinal calciotropic maternal hormone? *Trends in Endocrinology & Metabolism*. 2010;21:395-401.
- [25] Moor MB, Bonny O. Ways of calcium reabsorption in the kidney. *American Journal of Physiology-Renal Physiology*. 2016;310(11):F1337-50.
- [26] Riccardi D, Brown EM. Physiology and pathophysiology of the calcium-sensing receptor in the kidney. *American journal of physiology. Renal physiology*. 2010;298(3):F485-99.
- [27] Hall AK, Norman AW. Regulation of calbindin-D28K gene expression in the chick intestine: effects of serum calcium status and 1,25-dihydroxyvitamin D₃. *Journal of Bone & Mineral Research*. 1990;5(4):331-6.
- [28] Hannan FM, Walls GV, Babinsky VN, Nesbit MA, Kallay E, Hough TA, et al. The Calcilytic Agent NPS 2143 Rectifies Hypocalcemia in a Mouse Model With an Activating Calcium-Sensing Receptor (CaSR) Mutation: Relevance to Autosomal Dominant Hypocalcemia Type 1 (ADH1). *Endocrinology*. 2015;156(9):3114-21.
- [29] Motoyama HI, Friedman PA. Calcium-sensing receptor regulation of PTH-dependent calcium absorption by mouse cortical ascending limbs. *American Journal of Physiology. Renal Physiol*. 2002; 283(3):F399-406.
- [30] Topala CN, Schoeber JP, Searchfield LE, Riccardi D, Hoenderop JG, Bindels RJ. Activation of the Ca²⁺-sensing receptor stimulates the activity of the epithelial Ca²⁺ channel TRPV5. *Cell Calcium*. 2009; 45(4):331-9.
- [31] Hoenderop JG, Chon H, Gkika D, Bluysen HA, Holstege FC, St-Arnaud R, et al. Regulation of gene expression by dietary Ca²⁺ in kidneys of 25-hydroxyvitamin D₃-1 alpha-hydroxylase knockout mice. *Kidney International*. 2004;65(2):531-9.
- [32] Kumar R. Calcium metabolism. In: *The Principles and Practice of Nephrology*, edited by Jacobson HR, Striker GE, Klahr S, 2nd Ed., St. Louis, MO, Mosby-Year Book. 1995; 964–971.
- [33] Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. *American Journal of Physiology. Renal Physiology*. 2005;289: F8–F28.
- [34] Felsenfeld A, Rodriguez M, Levine B. New insights in regulation of calcium homeostasis. *Current Opinion in Nephrology & Hypertension*. 2013;22(4):371-6.
- [35] Tennakoon S, Aggarwal A, Kállay E. The calcium-sensing receptor and the hallmarks of cancer. *Biochimica et Biophysica Acta. Biophysica Acta*. 2016;1863:1398–1407.
- [36] Hu J, Spiegel AM. Structure and function of the human calcium-sensing receptor: insights from natural and engineered mutations and allosteric modulators. *J Cell Mol Med*. 2007;11:908-922.
- [37] Hendy GN, Canaff L. Calcium-Sensing Receptor Gene: Regulation of Expression. *Front. Physiol*. 2016;7:394. doi: 10.3389/fphys.2016.00394
- [38] Saidak Z, Brazier M, Kamel S, Mentaverri R. Agonists and allosteric modulators of the calcium-sensing receptor and their therapeutic applications. *Molecular Pharmacology*. 2009;76:1131–1144

- [39] Leach K, Gregory KJ. Molecular insights into allosteric modulation of class C G protein-coupled receptors. *Pharmacological Research*. 2017;116:105–118
- [40] Díaz-Soto G, Rocher A, García-Rodríguez C, Núñez L, Villalobos C. The Calcium-Sensing Receptor in Health and Disease. *Int Rev Cell Mol Biol*. 2016;327:321–369.
- [41] Conigrave AD, Ward DT. Calcium-sensing receptor (CaSR): pharmacological properties and signaling pathways, *Best Pract. Res. Clin. Endocrinol. Metab*. 2013;27:315–331.
- [42] Hofer A.M., Brown E.M. Extracellular calcium sensing and signalling. *Nature reviews. Molecular cell biology*. 2003;4(7): 530–538.
- [43] Acar S, Demir K, Shi Y. Genetic Causes of Rickets. *Journal of clinical research in pediatric endocrinology*. 2017;9(Suppl 2):88-105.
- [44] Xie R, Tang B, Yong X, Luo G, Yang S. Roles of the calcium sensing receptor in digestive physiology and pathophysiology (Review). *International Journal of Oncology*. 2014;45:1355-1362.
- [45] Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiological Reviews*. 2001;81:239-297
- [46] Kamitani-Kawamoto A, Hamada M, Moriguchi T, Miyai M, Saji, F, Hatamura I, et al. MafB interacts with Gcm2 and regulates parathyroid hormone expression and parathyroid development. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 2011;26(10): 2463–2472.
- [47] Han SI, Tsunekage Y, Kataoka K. GATA3 cooperates with GCM2 and MafB to activate parathyroid hormone gene expression by interacting with SP1. *Molecular and cellular endocrinology*. 2015;411:113–120
- [48] Liu Z, Yu S, Manley NR. Gcm2 is required for the differentiation and survival of parathyroid precursor cells in the parathyroid/thymus primordia. *Developmental biology*. 2007;305(1):333–346
- [49] Potts JT, Gardella TJ. Progress, paradox, and potential: parathyroid hormone research over five decades. *Annals of the New York Academy of Sciences*. 2007;1117: 196-208.
- [50] Hasan A, Pollak YE, Kilav-Levin R, Silver J, London N, Nechama M, et al. Kidney failure alters parathyroid Pin1 phosphorylation and parathyroid hormone mRNA binding proteins leading to secondary hyperparathyroidism. *bioRxiv* 2021.12.06.470227; doi: <https://doi.org/10.1101/2021.12.06.470227>
- [51] Kumar R, Thompson JR. The regulation of PTH secretion and synthesis. *JASN* 2011;22(2):216-224
- [52] Vaira V, Verdelli C, Forno I, Corbetta S. MicroRNAs in parathyroid physiopathology. *Mol Cell Endocrinol*. 2017;456:9-15. doi:10.1016/j.mce.2016.10.035
- [53] Loupy A, Ramakrishnan SK, Wootla B, et al. PTH-independent regulation of blood calcium concentration by the calcium-sensing receptor. *J Clin Invest*. 2012;122(9):3355-3367. doi:10.1172/JCI57407
- [54] Vahe C, Benomar K, Espiard S, Coppin L, Jannin A, Odou MF, et al. Diseases associated with calcium-sensing receptor. *Orphanet J Rare Dis*. 2017;12:19. doi: 10.1186/s13023-017-0570-z
- [55] Goltzman D, Hendy GN. The calcium-sensing receptor in bone— mechanistic and therapeutic insights. *Nat Rev Endocrinol*. 2015;11:298– 307. doi: 10.1038/nrendo.2015.30
- [56] Santa Maria C, Cheng Z, Li A, Wang J, Shoback D, Tu C-L, et al. Interplay between CaSR and PTH1R signaling in skeletal development and osteoanabolism. *Semin Cell Dev Biol*. 2016;49:11–23.
- [57] Quinn SJ, Ye CP, Diaz R, Kifor O, Bai M, Vassilev P, et al. The Ca²⁺-sensing receptor: a target for polyamines. *The American Journal of Physiology*. 1997;273(4):C1315-23
- [58] Tu CL, Celli A, Mauro T, Chang W. Calcium-Sensing Receptor Regulates Epidermal Intracellular Ca²⁺ Signaling and Re-Epithelialization after Wounding. *J Invest Dermatol*. 2019;139(4):919-929.
- [59] Das S, Clézardin P, Kamel S, Brazier Mand Mentaverri R. The CaSR in Pathogenesis of Breast Cancer: A New Target for Early Stage BoneMetastases. *Front. Oncol*. 2020;10:69.doi:10.3389/fonc.2020.00069
- [60] Yarova PL, Stewart AL, Sathish V, Britt RD, Thompson MA, P Lowe AP, et al. "Calcium-sensing receptor antagonists abrogate airway hyperresponsiveness and inflammation in allergic asthma". *Science Translational Medicine*. 2015;7 (284): 284ra60. doi:10.1126/scitranslmed.aaa0282.

- [61] Aggarwal A, Prinz-Wohlgenannt M, Tennakoon S, Höbaus J, Boudot C, Mentaverri R, et al. "The calcium-sensing receptor: A promising target for prevention of colorectal cancer". *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 2015;1853 (9): 2158–67.
- [62] Kelly JC, Lungchukiet P, MacLeod RJ. Extracellular calcium-sensing receptor inhibition of intestinal epithelial TNF signaling requires CaSR-mediated Wnt5a/Ror2 interaction. *Front. Physiol.* 2011;2:17. doi: 10.3389/fphys.2011.00017
- [63] Kim JY, Ho H, Kim N, Liu J, Tu CL, Yenari MA, Chang W. "Calcium-sensing receptor (CaSR) as a novel target for ischemic neuroprotection". *Annals of Clinical and Translational Neurology*. 2014;1(11):851–66.
- [64] Dal Prà I, Chiarini A, Armato U. "Antagonizing amyloid- β /calcium-sensing receptor signaling in human astrocytes and neurons: a key to halt Alzheimer's disease progression?". *Neural Regeneration Research*. 2015;10 (2): 213–8.