Genotype and phenotype correlation of calcium-sensing receptor variants

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Mutations of the calcium-sensing receptor (CaR) gene give rise to disorders of renal calcium excretion. It is now demonstrated that even common variants of CaR can cause alteration in tissue sensitivity to extracellular calcium and are associated with primary hypercalciuria. Agents targeting these functional domains may well be an option for the management of hypercalciuria and renal stones.


The extracellular calcium-sensing receptor (CaR) plays a key role in maintaining extracellular calcium homeostasis. It enables several tissues involved in this process to detect minute changes in extracellular Ca\(^{2+}\) and respond with an alteration in the cellular function to restore extracellular Ca\(^{2+}\) to the normal range. CaR is a member of group II within family C of the superfamily of G protein-coupled receptors. Structurally, CaR is a fusion protein that consists of an external milieu and transmembrane and intracellular domains that participate in signal transduction. CaR was initially cloned from the parathyroid gland and was subsequently identified in tissues that participate in the regulation of Ca\(^{2+}\) homeostasis, including calcitonin-secreting C cells of the thyroid gland, kidney cells, and also bone and intestinal cells. The sensing of low extracellular Ca\(^{2+}\) by CaR evokes parathyroid hormone (PTH) secretion by the parathyroid glands. PTH acts on the kidneys to mediate a number of physiological activities, including promotion of distal tubular calcium reabsorption, enhancement of phosphaturia, and stimulation of conversion of 25-hydroxyvitamin D\(_3\) to the active form 1,25-dihydroxyvitamin D\(_3\). Increased 1,25-dihydroxyvitamin D\(_3\) production enhances gastrointestinal calcium absorption, which synergizes with PTH to mobilize calcium release from the skeleton. Together with increased renal tubular calcium reabsorption, movement of calcium into the extracellular fluid from the gastrointestinal tract and bone helps to normalize extracellular Ca\(^{2+}\) levels.

CaR is expressed in various parts of the renal tubules, including the apical membrane of the proximal tubule, the basolateral membrane of the medullary thick ascending limb, the cortical thick ascending limb, the distal convoluted tubules, and the apical surface of the inner medullary collecting duct. In the kidney, CaR mediates several functions: it reduces the phosphaturic action of PTH in the proximal tubule, and it inhibits the reabsorption of salt in the medullary thick ascending limb and cortical thick ascending limb, and the reabsorption of calcium in the distal convoluted tubule. It also reduces the reabsorption of calcium and magnesium in the cortical thick ascending limb and inhibits vasopressin-stimulated water reabsorption in the inner medullary collecting duct to limit the urine-concentrating ability.

Since the first cloning of CaR in bovine parathyroid tissue, a number of inactivating and activating mutations of CaR have been described (Figure 1). Inactivating mutations of CaR, characterized by resistance to extracellular Ca\(^{2+}\), were shown to be associated with two hypercalcemic disorders, familial hypocalciuric hypercalcemia and neonatal severe primary hyperparathyroidism. Activating mutations of CaR, characterized by increased sensitivity to extracellular Ca\(^{2+}\), are linked to autosomal-dominant and sporadic hypocalcemia. Subjects with inactivating mutations of CaR exhibit absolute or relative hypocalciuria due to diminished hypercalcemia-induced calciuresis in the distal renal tubules, whereas subjects with activating mutations of CaR have reduced parathyroid set point and excessive renal calcium excretion despite hypocalcemia. These mutations and inherited disorders of CaR are rare but allow the understanding that the extracellular calcium ion can serve as an extracellular first messenger for CaR.

Perhaps more interesting are the recent observations of the association of common polymorphisms of CaR with renal calcium excretion in the general population. Vezzoli et al.\(^3\) (this issue) report that the single-nucleotide polymorphism at nucleotide position 2968 (amino acid position 990) is associated with primary hypercalciuria. Vezzoli et al.\(^3\) studied three single-nucleotide polymorphisms at the intracytoplasmic tail of CaR: G2956T, C3031G, and A2968G, which are in high linkage disequilibrium with each other (Figure 1). Their results show that the variant G allele of A2968G, which results in replacement of arginine with glycine (R990G), was associated with an increased risk of hypercalciuria. Women with primary hypercalciuria but a negative history of kidney stones had a higher frequency of the 990G variant allele, and homozygous or heterozygous carriers of this allele had a 5.2-fold increased risk of hypercalciuria when compared with homozygous carriers of the wild-type allele. Vezzoli et al.\(^3\) also confirmed that the 990G variant allele was associated with a gain of function of the CaR. This was evidenced by a lower extracellular Ca\(^{2+}\) concentration needed to produce the half-maximal intracellular Ca\(^{2+}\) response in human embryonic kidney (HEK-293) cells transfected with the 990G allele. With the use of the bioinformatics approach, it was predicted that the

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substitution of arginine with glycine would cause a significant alteration in the secondary structure of the CaR protein.

This 990G allele has previously been reported to be associated with hypercalciuria and calcium stone formation with or without the presence of primary or secondary hyperparathyroidism.4–9 although other studies failed to confirm these associations.10,11 It is not certain whether sporadic cases of primary hypercalciuria without stone formation, and hypercalciuria with nephrolithiasis with or without hyperparathyroidism, represent a continuum of one single disorder. Because of the low frequency of the 990G allele, most studies are unable to address the dose-dependent effect of this allele. In reports of hypercalciuria and nephrolithiasis with or without hyperparathyroidism, the 990G allele was associated with lower levels of PTH and/or serum calcium, suggesting that the variant receptor had increased sensitivity to extracellular Ca2+ in both parathyroid and kidney tubules. However, in the study by Vezzoli et al.,3 women carrying the 990G allele only had hypercalciuria with normal serum calcium and PTH levels. The variable response in serum calcium and PTH in these different reports suggests that the tissue sensitivity of 990G in the kidney tubules may differ from that in the parathyroid tissues. It remains to be confirmed whether the expression level of this 990G allele may differ between the kidney tubules and parathyroid cells, as well as between the bone and intestinal cells. It is also likely that this variant allele might affect interaction of CaR with different cofactors in the kidney tubules and parathyroid cells that modulate the downstream pathway to produce a variable phenotype and clinical outcome. Furthermore, as the studied phenotype is a 24-hour urine calcium concentration — representing a summation of the effect of CaR on different segments of the kidney tubules as well as other non-CaR-mediated factors such as dietary calcium intake and vitamin D status — the actual effect of 990G on distal tubular calcium absorption may be difficult to detect.

With the recent advances in genetic epidemiology and bioinformatics, a better understanding of the pathogenesis of common disorders as well as a more accurate prediction of treatment responses is possible. Preliminary studies in end-stage renal failure patients have pointed to an improved response in carriers of 990G to the calcimimetic drug cinacalcet in terms of PTH suppression.12 It remains to be confirmed whether an inhibitory agent that specifically targets position 990 would reduce hypercalciuria and inhibit renal stone formation.

REFERENCES