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FAMILIAL HYPOCALCIURIC HYPERCALCEMIA AND CALCIUM SENSING RECEPTOR

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SUMMARY –Familial hypocalciuric hypercalcemia (FHH) is a lifelong, benign autosomal dominant disease characterized by hypercalcemia, normal to increased parathyroid hormone level, and a relatively low renal calcium excretion. Inactivation of the calcium-sensing receptor in heterozygous patients results in FHH, while in homozygous patients as well as in compound heterozygous or dominant negative heterozygous patients, it may result in neonatal severe hyperparathyroidism (NSHPT). Parathyroid surgery is not indicated in FHH and does not lower plasma calcium unless total parathyroidectomy is performed, in which case hypocalcemia ensues. There is currently no definitive medical treatment available, although pamidronate can be used to stabilize these patients before parathyroidectomy. Some NSHPT patients are asymptomatic subsequently in their lives. In this paper, clinical characteristics of this relatively rare disorder are presented.

Key words: *Hypercalcemia, congenital; Receptors, calcium sensing; Hypercalcemia – drug therapy*

The Role of Calcium in the Human Body

Many physiological processes in the human body use either intracellular or extracellular Ca^{2+}. Intracellular Ca^{2+} exists in the cytosol of all cell types, working as a second messenger and enzyme cofactor. It coordinates and controls, i.e. modulates, various cell functions including muscle contractions, hormone secretion, glycogen metabolism, cell differentiation and proliferation, cell motility, and nerve cell function[1].

Under normal conditions, the intracellular concentration of Ca^{2+} is 100 nmol/L, i.e. much less than in the extracellular phase. The concentration of intracellular Ca^{2+} fluctuates by about 1 mmol/L upon cell activation, either due to the release of Ca^{2+} from intracellular stores or because of the increased calcium influx, but also due to the calcium-induced calcium release from the sarcoplasmic reticulum. The concentration of extracellular Ca^{2+} (i.e. blood levels) remains virtually constant due to the sensitive homeostatic mechanism[1].

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Under normal conditions, the plasma level of calcium is highly regulated through the calciotropic hormones, i.e. parathyroid hormone (PTH), 1,25-dihydroxy-vitamin D, and to a lesser extent calcitonin. PTH is the most important and fastest regulator of the calcium level in serum. The cells of the parathyroid glands are Ca^{2+} sensitive and respond to even small and transient changes of the extracellular Ca^{2+} concentration. PTH increases the release of Ca^{2+} from the bones, enhances distal tubular Ca^{2+} reabsorption of the kidneys, and simultaneously reduces phosphate reabsorption[1]. It is worth noticing that increased $[Ca^{2+}]i$ inhibits PTH secretion in contrast to most other signaling pathways where increased $[Ca^{2+}]i$ stimulates secretion of the relevant hormone.

The Role of the Calcium-Sensing Receptor in Calcium Homeostasis of Humans

For a long time, one has expected the existence of a receptor closely regulating the level of calcium in humans. The calcium-sensing receptor (CaSR) in humans was detected by the expression cloning technique by Brown *et al.* in 1993[2]. There has been

an explosion of interest in the CaSR since it was first cloned. The CaSR is a glucoprotein belonging to the Family C II of the superfamily of G-protein coupled receptors. Members of the Family C II receptors are expressed both centrally and in peripheral tissues.

All these receptors are, in general, composed of 4 main protein domains:

1) an atypically large hydrophobic N-terminal extracellular, nutrient binding, Venus Flytrap (VFT) domain;
2) cysteine-rich domain that couples nutrient binding to receptor activation;
3) 7-transmembrane domain (TMD), which is involved in the process of ligand-induced signaling and G-protein activation; and
4) intracellular C-terminal signaling domain (ICD), which is required for the activation of intracellular signaling pathways[3,4].

Recent studies have demonstrated that the CaSR forms disulfide-linked dimers[5]. CaSR couples to phosphatidylinositol (PI) specific phospholipase C and induces mobilization of intracellular Ca^{2+}. This explains why elevated concentrations of extracellular Ca^{2+} and Mg^{2+} rapidly induce intracellular Ca^{2+} mobilization and inositol phosphate turnover in parathyroid cells *via* G-protein dependent activation of PI-specific phospholipase C. Ca^{2+}, and to a lesser degree Mg^{2+}, is bound to the extracellular N-terminal part of the CaSR. Ca^{2+} is released from the endoplasmic reticulum, and simultaneously the influx of extracellular Ca^{2+} is increased through voltage-independent calcium channels. Thus, a cascade of intracellular changes is initiated mediating an effect corresponding to the recorded level of P-Ca^{2+}[3,6].

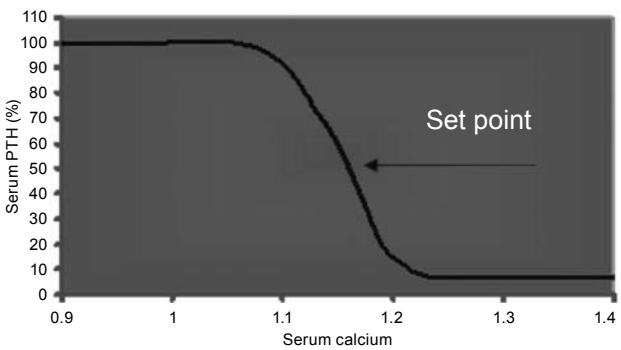

Fig. 1. The calcium se-point

CaSR is found in the chief cells of the parathyroid glands, the C-cells of the thyroid gland, the renal cells, the gut and the bones. In the gut, CaSR seems to be involved in sensing of amino acids, and in the bones CaSR inhibits the formation and activity of osteoclasts and stimulates osteoblasts. The C-cells of the thyroid gland increase calcitonin secretion[4].

The main function of the CaSR is the regulation of the synthesis and secretion of PTH by the parathyroid glands. By means of the CaSR, the cells are able to sense local changes in the concentration of Ca^{2+} and thereby change their functions. An increase in Ca^{2+} leads to the activation of CaSR resulting in a decrease in PTH secretion. On the other hand, a decrease in the serum Ca^{2+} level inactivates the CaSR that evokes an increase in PTH secretion within few seconds or minutes[6].

The concentration of extracellular Ca^{2+} resulting in 50% inhibition of the maximal PTH-secretion is referred to as the calcium set-point. The calcium set-point reflects the sensitivity of the CaSR to extracellular Ca^{2+}. A large number of mutations in the gene coding for the CaSR have been demonstrated to affect the calcium set-point (see Fig. 1)[7].

Polymorphisms are common variations of DNA. In contrast to a mutation, it is defined as a non-pathogenic change, but in some extremely rare cases it can cause mild disease. In some cases, polymorphisms might affect the set-point of the receptor, but often to a very small degree, and thus discrete or intermittent hypercalcemia can be induced. These patients are often identified in relation to diagnosing hypercalcemia.

In kidneys, CaSR is expressed in all nephron segments (see Table 1), with the possible exception of the glomeruli, where its presence is debated[8].

The functionally important mutations in the CaSR lead to changes in the calcium homeostasis and to diseases. Mutations in the gene coding for the CaSR can cause disruptions of the calcium homeostasis and eventually a disease, since the mutation can either activate or inactivate CaSR. Activation of the receptor can result in autosomal dominant hypocalcemia (see Table 1). On the other hand, inactivation of the receptor can cause familial hypocalciuric hypercalcemia (FHH) in heterozygous patients (see Table 1). Different mutations cause hypercalcemia of various degrees of clinical severity (see Table 2)[9]. Inactivation of the receptor in homozygous patients, as well as in compound heterozygous or domi-

Table 1. Renal effects of calcium-sensing receptor (CaSR)[8]

Nephron segment	Effect of CaSR
Juxtaglomerular apparatus	– CaSR activation inhibits renin secretion by reducing cAMP synthesis[8]
Proximal tubule	– CaSR activation results in an antiphosphaturic effect[8]
Thick ascending loop of Henle (TALH)	– CaSR stimulation may have an inhibitory effect on sodium/potassium/chloride carrier (NKCC2) activity *via* several mechanisms – CaSR activation diminishes Ca^{2+} and Mg^{2+} – CaSR also inhibits low-conductance potassium channels (ROMK) activity and sodium-potassium pump activity, and PTH-stimulated calcium reabsorption in the cortical TALH[8]
Distal convoluted tubule	– CaSR inhibits active calcium reabsorption mediated by the basolateral calcium pump (PMCA) through protein kinase C activation – CaSR also reduces potassium flux through the potassium channels by interacting directly with them. The drop in potassium efflux has a negative effect on sodium-potassium pump activity and reduces sodium reabsorption[8]
Collecting duct	– These cells express water channels of type 2 (AQP-2), but also AQP-3 and 4 – Vasopressin binding to their V2 receptor leads to AQP-2 insertion in the luminal membrane and elicits antidiuresis. CaSR is expressed on the luminal membrane and antagonizes vasopressin activity by altering AQP-2 trafficking, thus reducing urine concentration capacity[8]. This inhibition of maximal urinary concentrating capacity may have physiologic relevance as a means of avoiding excessive levels of Ca^{2+} in the distal urinary collecting system that might otherwise predispose to renal stone formation during times of antidiuresis[10]

nant negative heterozygous patients, results in neonatal severe primary hyperparathyroidism (NSHPT)[10].

At least 64 inactivating mutations have previously been described in the CaSR gene. Christensen *et al.* have recently described 22 inactivating mutations, of which 19 are novel. A significant phenotype difference (e.g., difference in the degree of hypercalcemia) was detected among individual mutations[11].

Familial Hypocalciuric Hypercalcemia

In 1966, FHH was described for the first time and was named familial HPT by Jackson and Boonstra[7]. Furthermore, in 1972, the disease was characterized and named familial benign hypercalcemia by Foley because the disease is generally asymptomatic and does not require treatment, in contrast to the homozygous disease NSHPT[7,12]. Finally, in 1977, the condition was termed FHH by Marx *et al.*[13]. The cause of the disease was first known in 1993, when CaSR in humans was detected by the expression cloning technique by Brown *et al.*[2]. Familial hypocalciuric hypercalcemia is an autosomal dominant disease with 100% penetrance. Since the disease is autosomal dominant, half of an offspring will statistically inherit the disease, and because of the high penetrance, hypercalcemia will be observed in all

Table 2. Disease related to calcium-sensing receptor (CaSR)9

Activating mutation	Inactivating mutation
Autosomal dominant hypocalcemia (ADH)	Familial hypocalciuric hypercalcemia (FHH)
Idiopathic hypercalciuria (IH)	Neonatal severe hyperparathyroidism (NSHPT)
Idiopathic epilepsy (IE)	Expression in a kindred of FHH or NSHPT affected members (FHH/NSHPT)
Bartter`s syndrome type V	Familial isolated hyperparathyroidism (FIHP)
	Tropical chronic pancreatitis (TCP)

heterozygous patients with FHH. Hypercalcemia can be observed in these patients at all ages, even during the first week of their lives[13]. The prevalence of FHH is approximately 1:10,000[7]. The gene responsible for FHH was at first mapped to the long arm of chromosome 3 (FHH1). Locus on this chromosome has been documented to be the gene encoding the CaSR. A phenotypically similar disorder has been linked to 2 different loci (short arm = FHH2 and long arm = FHH3) on chromosome 19. FHH3 is also referred to as the Oklahoma variant[14]. FHH patients are usually asymptomatic, but their biochemical features are very similar to primary hyperparathyroidism (PHPT), which is why these can easily be confused. However, it is important to differentiate between FHH and PHPT because the prognosis and treatment differ[15]. PHPT is characterized by hypercalcemia, hypercalciuria, enlarged parathyroid glands and significantly increased concentration of serum PTH. PHPT is treated efficiently with parathyroidectomy[7,16]. FHH is characterized by hypercalcemia, relative hypocalciuria and inappropriately normal to high levels of PTH[10]. The increased serum PTH concentration in relation to the patient's hypercalcemia reflects an altered calcium set-point[7]. Furthermore, FHH patients are characterized by a moderately increased serum magnesium level and normal serum phosphate level[6]. There are significant similarities between the characteristics of these diseases.

Christensen *et al*. used the Receiver Operating Characteristics (ROC) analysis and an overlap analysis to demonstrate that the calcium/creatinine clearance ratio (CCCR) was marginally better in differentiating between FHH and PHPT compared to other estimations of renal calcium excretion. CCCR is generally less than 0.01 in patients with FHH and higher than 0.02 in patients with PHPT. Unlike PHPT, most patients with FHH do not have osteoporosis, renal function deficits, ulcer disease, or increased risk of cardiovascular disease[16]. In the clinical setting, the distinction between FHH and PHPT is often based on the CCCR in hypercalcemic patients with clinical suspicion of PHPT or FHH. A two-step diagnostic procedure has been proposed, where the first step is based on the CCCR with a cut-off at <0.02, and the second step is CaSR gene analysis in patients with FHH or PHPT[16]. Christensen *et al*. demonstrated FHH patients to have normal 25-OH-vitamin-D, but increased 1,25-$(OH)_2$-vitamin-D (compared with population based sex-, age- and season-matched normal controls). They also concluded that inactivating CaSR mutations do not cause deleterious effects on bone as evaluated by DXA measurements, in spite of increased plasma levels of PTH and alkaline phosphatase compared to normal control[11,17].

Familial hypocalciuric hypercalcemia cannot be cured and is resistant to partial parathyroidectomy, since the condition is due to a general defect in the CaSR throughout the body and not only localized to the parathyroid glands. Total parathyroidectomy results in hypocalcemia[7].

Complications reported by FHH patients are recurring pancreatitis and chondrocalcinosis. In addition, there is a risk of inappropriate parathyroidectomy. There are uncertainties as to the occurrence of osteoporosis, myopathy, nephrolithiasis, diabetes and hypertension in FHH patients. FHH should be treated if patients have severe symptoms[7]. Recent studies show that cinacalcet (Mimpara®) can restore calcium sensitivity of the parathyroid glands and treatment with this agent might be useful in preventing complications of FHH[18].

Essentially, the patients' lists of prescribed drugs must be revised since several drugs affect the metabolism of calcium. FHH patients with osteoporosis must take vitamin D supplement, since it has a better effect on preventing fractures and preserving muscle function than calcium supplementation. A few FHH patients have developed PHPT. Thus, annual measurement of serum Ca^{2+} and PTH is recommended[7].

Moreover, family screening is important in order to avoid unnecessary parathyroidectomy in patients with asymptomatic hypercalcemia. Furthermore, measurement of Ca^{2+} in family members is indicated if differentiation between FHH and PHPT in the proband is difficult[7].

Conclusion

Although the disease has been known since 1966, we still know very little about it and further research is required. FHH is easily confused with milder cases of the more common PHPT, which is generally treated by parathyroidectomy. In the case of FHH, parathyroidectomy is not only unnecessary

but also inappropriate, since it does not cure FHH-associated hypercalcemia. It is therefore important to identify patients with FHH to prevent unnecessary parathyroidectomy. Recent studies show that cinacalcet (Mimpara®) can restore calcium sensitivity of the parathyroid glands and treatment with this agent might be useful in preventing complications of FHH. Since most cases of FHH are associated with loss-of-function mutations in a single gene *(CASR)*, genetic testing can assist in the diagnosis of FHH. Genetic testing for FHH-associated mutations in CaSR can help prevent unnecessary and inappropriate parathyroidectomy in patients with FHH.

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Sažetak

OBITELJSKA HIPOKALCIURIČNA HIPERKALCEMIJA I RECEPTOR OSJETLJIV NA KALCIJ

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Obiteljska hipokalciurična hiperkalcemija je relativno čest uzrok asimptomatske hiperkalcemije. Obiteljska hipokalciurična hiperkalcemija je autosomno dominantni poremećaj obilježen umjerenom hiperkalcemijom i relativnom hipokalciurijom. Obiteljska hipokalciurična hiperkalcemija nastaje uslijed inaktivirajuće mutacije gena za CaSR, receptor osjetljiv na kalcij. Najčešće ovaj poremećaj ne zahtijeva kirurško liječenje, kako je to slučaj s drugim oblicima primarnog hiperparatireoidizma. Pokušaji da se hiperkalcemija liječi lijekovima nisu bili uspješni i nisu snizili razinu kalcija u serumu. Studije pokazuju da sinakalcet (Mimpara®) može zaustaviti nenormalno prepoznavanje serumskog kalcija od strane paratireoidnih žlijezda, tako da dođe do normalne sekrecije paratireoidnog hormona. U ovom radu opisane su kliničke manifestacije ove relativno rijetke bolesti.

Ključne riječi: *Hiperkalcemija, prirođena; Receptori osjetljivi na kalcij; Hiperkalcemija – farmakoterapija*