Calcium homeostasis and disorders of the calcium-sensing receptor

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Overview of calcium homeostasis.

In health, the serum ionised calcium concentration (Ca\(^{2+}\)) is tightly regulated within the range 1.1–1.4 mmol/l by the actions of the major calciotropic hormones, parathyroid hormone (PTH) and 1,25–dihydroxyvitamin D\(_3\) (1,25(OH)\(_2\)D\(_3\)). Any decrease in the extracellular calcium ion concentration leads to an increase in the rate of release from the parathyroid chief cells of PTH, which acts via the PTH receptor to increase the distal renal tubular re-absorption of calcium within minutes. PTH secretion also enhances the activity of osteoclasts and other bone cells, causing calcium release from the skeleton within 1–2 hours. More prolonged PTH release stimulates 1\(\alpha\)-hydroxylase activity in the proximal tubular cells which leads to 1,25(OH)\(_2\)D\(_3\) production. The latter has long-term effects, regulating both intestinal calcium absorption and skeletal calcium turnover over days to weeks. All these mechanisms act to produce an increase in the serum Ca\(^{2+}\), correcting it towards the baseline value, then completing the ‘feedback loop’ by inhibiting PTH release. The increased 1,25(OH)\(_2\)D\(_3\) levels also directly inhibit transcription of the PTH gene.

Role of the calcium-sensing receptor

The calcium-sensing receptor (CaR) is a G protein-coupled receptor which allows the parathyroid chief cells, the renal tubular epithelial cells and the thyroidal C cells, to respond to changes in the extracellular calcium concentration. The ability of the CaR to sense the serum Ca\(^{2+}\) is essential for the appropriate regulation of PTH secretion by the parathyroids. Calcitonin secretion and renal tubular calcium reabsorption are also directly regulated by the action of Ca\(^{2+}\) on the CaR. The CaR gene is located on chromosome 3q13–q21 and encodes a 1,078 amino acid protein with a large extracellular domain and seven transmembrane domains. The CaR is expressed in many tissues, including brain, lung, ileum, pituitary and tests, as well as parathyroid, kidney and thyroid C cells. Three common human disorders are due to abnormalities of the CaR gene:

- familial benign hypocalciuric hypercalcaemia (FBHH)
- neonatal severe hyperparathyroidism (NSHPT)
- autosomal dominant hypocalcaemia with hypercalcuria (ADHH).

Familial benign hypocalciuric hypercalcaemia

FBHH is an autosomal dominant disorder characterised by lifelong and generally asymptomatic hypercalcaemia. It can be difficult to distinguish this condition from primary hyperparathyroidism (PHP) and FBHH is the diagnosis in about 10% of subjects who have successful parathyroid exploration. The hypercalcaemia is manifest as early as the first week of life in affected subjects, but may vary in severity from borderline elevation of ionised calcium alone to marked hypercalcaemia (total serum calcium ≥3.5 mmol/l). Borderline hypermagnesaemia (0.95–1.10 mmol/l) is also found in over half the cases. Serum phosphate values are normal or slightly reduced. Urinary calcium excretion is generally in the low normal to reduced range, with 75% of patients having 24-hour calcium excretion less than 2.5 mmol and 95% with values less
than 5.0 mmol\(^2\). This low normal or frankly low urinary calcium excretion is inappropriate since these subjects are hypercalcaemic and this contrasts to the elevated values generally seen in patients with PHP.

The urinary calcium clearance (Cacl) to creatinine clearance (Crcl) ratio can be used to improve the discrimination of FBHH from PHP (see Box 1). More than 80% of patients with FBHH have a Cacl/Crcl ratio below 0.01, whereas more than 70% of those with PHP have values above this cut-off\(^7,8,10\). Although of some diagnostic help, a low Cacl/Crcl ratio is neither pathognomonic of FBHH nor does it exclude PHP. Serum PTH in FBHH is generally within the normal range, but up to 20% of affected subjects have mildly elevated PTH\(^8,10\). Serum 25-hydroxyvitamin D and 1,25(OH)\(_2\)D\(_3\) levels are within the normal range. The vast majority of subjects with FBHH are asymptomatic\(^7–10\), but acute pancreatitis\(^7,11\), articular chondrocalcinosis and gallstones\(^8\) have been reported to occur more commonly in some series of FBHH patients. Paradoxically, there have also been occasional reports of calcium nephrolithiasis in FBHH\(^7\).

A diagnosis of FBHH should lead to a conservative management strategy. The patient should be warned about the hazards of inappropriate neck surgery in the future, and should not be placed on a low calcium diet. Approximately two-thirds of the FBHH kindreds have unique heterozygous mutations of the CaR gene (Fig 1)\(^6,12,13\). Many of these are missense mutations (ie amino acid substitutions).

**Box 1.** Calculation of calcium clearance (Cacl) to creatinine clearance ratio (Crcl).

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\text{Cacl/Crcl} = \frac{\text{urinary calcium x plasma creatinine}}{\text{plasma calcium x urinary creatinine}}
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**Notes:**
- A spot urine sample with a synchronous venous blood sample is required.
- Increased reproducibility will be obtained if subjects undergo an overnight fast (with free access to water) and the second voiding of the morning is sampled.
- All plasma and urine values used for the calculation must be in the same units (eg mmol/l).
that tend to cluster within the extra-cellular domain of the receptor\textsuperscript{12,13}. \textit{In vitro} expression studies of the CaR mutations found in FBHH have demonstrated either a relative loss of function or total inactivation of the CaR. Thus, loss of function CaR mutations cause an increase in the set point for serum Ca\textsuperscript{2+}-responsive PTH release, and elevate the renal calcium reabsorption at any given level of serum calcium, leading to the hypercalcaemia and hypocalciuria found in FBHH\textsuperscript{6,12,13}. The remaining one-third of FBHH families who do not have a mutation within the coding region of the CaR may have either an abnormality in the non-coding regions of the gene or a mutation at one of the uncharacterised FBHH loci located on chromosome 19\textsuperscript{6}. This genetic heterogeneity currently precludes the use of mutational analysis for diagnostic purposes in FBHH.

**Neonatal severe hyperparathyroidism**

NSHPT can be defined as the occurrence of symptomatic hypercalcaemia in the first six months of life, associated with bony signs of hyperparathyroidism\textsuperscript{9}. However, it is generally manifest in the first few \textit{days} of life, with failure to thrive, hypotonia, constipation and respiratory distress. It is often a life-threatening disorder, with a mortality of over 25\% in historical series\textsuperscript{9,10,14}. Bony abnormalities are marked, and include:

- underminerisation
- sub-periosteal erosion and metaphyseal destruction of the long bones
- multiple fractures of both the long bones and ribs
- rib-cage deformity
- craniotabes.

There may be dramatic hypercalcaemia, with total serum calcium values in the 5.0–7.0 mmol/l range, although some NSHPT infants have a lesser degree of hypercalcaemia. Circulating PTH is elevated (often over five times higher than the normal range), with normal or low serum phosphate, normal or elevated serum magnesium levels, elevated bone alkaline phosphatase, and an inappropriately normal or frankly low urinary calcium excretion.

Treatment of children with severe NSHPT should be removal of all parathyroid tissue in the first month or two of life, since delay leads to increasingly severe bony and rib-cage abnormalities, with death from emaciation and respiratory failure\textsuperscript{9,10}. A trial of conservative treatment is warranted in infants with milder hypercalcaemia who are thriving. In severe cases, parathyroid histology shows four-gland hyperplasia with the total parathyroid weight typically 10–20 times normal\textsuperscript{14}. After the infant is rendered parathyroid, there is a dramatic fall in the serum calcium, necessitating vitamin D and calcium therapy. The constitutional symptoms quickly reverse, with resolution of the bony abnormalities over about six months\textsuperscript{9,10,14}.

The majority of NSHPT children are born to normocalcaemic parents and appear to be sporadic cases, but NSHPT is now recognised to occur in a small proportion of children of FBHH families. Mutational analysis of the CaR has confirmed that severe NSHPT in the offspring of consanguineous FBHH parents is due to homozygous CaR mutations\textsuperscript{6,13}. However, three patients with sporadic NSHPT have been reported with \textit{de novo} heterozygous CaR mutations\textsuperscript{12}, suggesting that a substantial number of cases of sporadic hypercalcemia in infancy may be due to such \textit{de novo} CaR mutations.

**Autosomal dominant hypocalcaemia with hypercalciuria**

As it became clear that loss of CaR function caused FBHH, it was speculated that inappropriate activation of the CaR could lead to hypocalcaemia with hypercalciuria. Investigation of kindreds with what appeared to be autosomal dominant forms of hypoparathyroidism identified such ‘activating’ CaR muta-
Some affected individuals from these families were asymptomatic, but others had suffered seizures, generally occurring spontaneously in the neonatal period or before the age of three years in association with a febrile illness. Other symptoms such as tetany or muscle cramps have been reported in some members of these kindreds\textsuperscript{15,16}. It is important to distinguish the ADHH syndrome from true idiopathic hypoparathyroidism (IHP)\textsuperscript{17} because subjects with ADHH are particularly susceptible to the development of nephrocalcinosis, nephrolithiasis and renal impairment during vitamin D treatment\textsuperscript{15}. These disorders may be differentiated on three biochemical grounds:

1. ADHH subjects almost invariably have significant hypomagnesaemia, in the 0.5–0.7 mmol/l range, lower than that typically seen in IHP.

2. Affected subjects generally have detectable levels of PTH at presentation. During treatment with vitamin D, the PTH levels become suppressed at, or below, the limit of detection.

3. ADHH subjects have, on average, higher urinary calcium excretion than those with IHP, with pre-treatment urine calcium to creatinine values above 0.15 mmol/mmol (in the presence of hypocalcaemia) being suggestive of ADHH\textsuperscript{15,16}.

Clinical differentiation between ADHH and IHP will not be possible in some cases, but other observations may also be helpful in establishing the diagnosis. During treatment with vitamin D analogues, ADHH subjects sometimes complain of thirst and polyuria when their serum calcium is in the normal range. The demonstration of a urinary concentrating defect in this situation, in the absence of glycosuria, may be considered a pathognomonic feature of ADHH: it is direct evidence of a decrease in the set point for calcium-responsive urinary concentration. Serum 1,25(OH)\textsubscript{2}D\textsubscript{3} levels are within the normal range, which contrasts with the low levels found in IHP. Finally, if vitamin D has been administered, patients with ADHH tend to be relatively refractory to changes in serum calcium. On a small dose of 1\alpha-calciol or calcitriol (0.25–0.5 \mu g daily), typically there will be little change in the serum Ca\textsuperscript{2+}, but a dramatic increase in urinary calcium concentration.

Asymptomatic subjects with ADHH require no treatment. Small doses of vitamin D analogues may be warranted on a short-term basis for ADHH subjects with recurrent seizures, the aim being symptomatic control rather than achieving normocalcaemia. If ADHH subjects, or those hypocalciuric in whom a diagnosis cannot be firmly established, are treated with vitamin D analogues, the urinary calcium excretion, urinary concentrating ability, renal function and renal ultrasound should be monitored at regular intervals.

More than 80\% of the reported ADHH kindreds have mutations of the CaR and in vitro studies have confirmed that these mutations produce a gain of CaR function\textsuperscript{15,16}. De novo mutations of the CaR have also been found in some children with sporadic hypocalcaemia\textsuperscript{18}, so ADHH may account for a substantial proportion of cases of what would previously have been classified as ‘sporadic idiopathic hypoparathyroidism’. It is important to try to distinguish these two disorders by clinical or molecular genetic means because of the high incidence of iatrogenic renal complications found in ADHH subjects inadvertently treated with vitamin D or its analogues.

Summary
The identification of the CaR has cast light on many aspects of normal extracellular calcium homeostasis, particularly in relation to the regulation of PTH and calcitonin secretion and renal calcium reabsorption. In addition, loss and gain of function CaR mutations have provided an insight into the pathogenesis of the clinical syndromes of FBHH, NSHPT and ADHH. Careful attention to the urinary calcium excretion, together with other investigations, should enable the physician to distinguish these disorders of calcium sensing from other causes of hypercalcaemia and hypocalcaemia.

References
Osteoporosis

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Osteoporosis is a systemic skeletal disease characterised by low bone mass and micro-architectural deterioration, with a consequent increase in bone fragility and susceptibility to fracture. A diagnosis of osteoporosis is made if the patient sustains a low trauma fracture or if bone mineral density (BMD) is more than 2.5 standard deviations (SD) below the mean for young people (Fig 1).

Pathophysiology

Bone undergoes a continual process of resorption and formation in discrete bone remodelling units, with about 10% of the adult skeleton remodelled each year. This turnover prevents fatigue damage, and is important in maintaining calcium homeostasis. Bone loss results from an imbalance between the rates of resorption and formation.

The human skeleton comprises about 80% cortical bone and 20% trabecular bone, which is more metabolically active. Osteoporotic fractures tend to occur at sites with more than 50% trabecular bone. Bone loss leads to thinning, and sometimes perforation, of the trabecular plates. The resulting change in architecture leads to a loss of strength disproportionate to the amount of bone lost.

Figure 1. Bone mass across life. The reference range is represented as mean ±2 standard deviations (SD). The broken line indicates the diagnostic threshold for osteoporosis according to the World Health Organisation criteria (2.5 SD below the young adult mean). Peak bone mass is achieved by the age of 30 years. After skeletal maturity, bone is lost in both sexes at a rate of about 1% per year, and women experience a phase of accelerated bone loss for three years after the menopause. Various factors may affect the rate of bone loss (eg calcium intake, exercise) (BMD = bone mineral density).