Introduction

In 1966, Jackson and Boonstra described a novel hypercalcemic syndrome in an index case and in 17 other members of the family spanning 3 generations that was inherited in an autosomal dominant pattern (1). Three and a half gland parathyroidectomy in the index case and removal of two parathyroid glands in two siblings failed to normalize serum calcium levels. A few years later, Foley et al. gave the syndrome a name, familial benign hypercalcemia, summarizing its key features as asymptomatic familial hypercalcemia with low or normal urinary calcium excretion. Other typical characteristics include normal parathyroid hormone (PTH) levels in the presence of hypercalcemia, high normal or slightly elevated serum magnesium, normal or slightly decreased serum phosphate, and normal parathyroid gland pathology (2). Almost three decades before the definition of the molecular basis of the syndrome, these authors astutely commented on relative hypocalciuria as being the hallmark of that entity. Moreover, they speculated that “the primary defect would be an abnormally high parathyroid gland reference input value for extracellular fluid calcium ion concentration” (i.e., abnormal sensing of extracellular calcium). Subsequently, several groups have described kindreds with a similar phenotype, commonly using the terms familial benign hypercalcemia (FBH) and familial hypocalciuric hypercalcemia (FHH), (3–8). Other descriptions include familial hypercalcemia and familial parathyroid hyperplasia. In this review, we will use a unifying term describing the key features of the syndrome, familial benign hypocalciuric hypercalcemia (FBHH) (6). A closely related familial disorder, neonatal severe hyperparathyroidism (NSHPT), was described 30 yr earlier (9) and was first associated with FBHH by Spiegel et al. (10). Index cases not uncommonly belong to FBHH kindreds and, in some cases, are the product of consanguineous marriages (11–17). NSHPT is characterized by severe hypercalcemia, failure to thrive, respiratory distress, and skeletal abnormalities. In its severest form, it represents the phenotype of inheriting a double dose of the abnormal FBHH gene.

Genetics of FBHH

A close examination of many FBHH pedigrees reveal that it is an autosomal dominant condition and that both genders are equally affected with over 90% penetrance (1–6,8,18–20). De novo mutations are extremely rare (21) and would be hard to recognize in view of the paucity of symptoms associated with the syndrome (see section Phenotype of FBHH). Several candidate genes involved in calcium homeostasis, including the MEN-1, MEN-2, and PTH...
genes, have been evaluated with negative results (22–24). In over 90% of families suitable for genetic analyses, the disease gene was linked to chromosome 3, otherwise called FBHH3q (25–28). Two rarer forms of the disease were linked to chromosome 19, FBHH19p and FBHH19q, otherwise called FBHHOK (27,29,30).

**Molecular Basis of FBHH3q: Mutations in the Calcium-Sensing Receptor**

Chou et al. first demonstrated the linkage of FBHH to chromosome 3 (25) and shortly thereafter Brown et al. cloned the bovine parathyroid calcium-sensing receptor (CaSR) (26). Because FBHH constitutes an experiment of nature exhibiting abnormal sensing of extracellular calcium, the CaSR was the natural candidate gene to pursue as the disease gene for that syndrome. Indeed, Pollak et al. quickly proceeded to demonstrate three different missense mutations in the CaSR gene in three separate families with FBHH (31). Since then, several investigators have demonstrated that in over 90% of affected FBHH families, the trait links to the region of chromosome 3 that contains the CaSR (28,31–35). In over two-thirds of these families, there are mutations of various types in the CaSR, and in the remainder, there may be CaSR mutations outside the coding region.

The CaSR is expressed in many tissues, including lung, intestine, brain, and thyroid C cells, but it is most heavily expressed in the parathyroid glands and the cortical thick ascending limb of the kidney (36). The predicted topology of the human CaSR protein is shown in Fig. 1. It has three main structural domains:

1. A large 612-amino-acid extracellular amino-terminal domain (ECD)
2. A 250-amino-acid serpentine seven transmembrane-spanning domain (TMD) that characterizes the superfamily of G-protein-coupled receptors (GPCRs)
3. An intracytoplasmic 222-amino-acid carboxy terminal tail

Several subfamilies of GPCRs share the CaSR’s large ECD, although their sequence similarity to the CaSR is modest (20–30% overall amino acid identity). These GPCRs are designated as belonging to the family of C receptors (37). They comprise three groups of receptors: (1) the metabotropic glutamate receptors that have as their ligands, glutamate, the major excitatory neurotransmitter in the central nervous system, (2) the CaSR and a family of putative pheromone (environmental cues) or odorant receptors, and (3) the GABAB receptors that have as their ligand, GABA (gamma amino butyric acid), the major inhibitory neurotransmitter in the central nervous system.

Activation of a normal calcium receptor suppresses PTH secretion and enhances urinary calcium excretion. Reduction or loss of function of a normal allele is therefore expected to result in inappropriate PTH secretion for the serum calcium level and relative hypocalciuric, the typical phenotype in FBHH. Most of the inactivating mutations in the CaSR are single amino acid substitutions (i.e., missense mutations); however, nonsense mutations, leading to truncated receptors (28) and insertions (e.g., of an Alu element), have also been described (34). To date, over 30 different mutations in the FBHH gene have been described (Fig. 1). Most of the mutations have been nonsense mutations occurring in the three regions of the CaSR as follows:

1. Within the first 300 amino acids of the ECD in about two-thirds of the cases
2. In the region of the ECD proximal to the first transmembrane domain
3. Within the transmembrane domains, extracellular or intracellular loops or in the carboxy terminal tail

A Swedish family with the first described point mutation in the cytoplasmic tail of the receptor exhibits a phenotype that is a hybrid between FBHH and hyperparathyroidism (38).

Heath et al. described so-called “silent benign polymorphisms” within the carboxy-terminal part of the CaSR that occurred in up to one-third of some 100 unaffected subjects that were studied (35). The three clustered CaSR polymorphisms occurred in exon 7 as follows: 986Ala/Ser (A986S), 990 Arg/Gly (R990G), and 1011 Gln/Glu (Q1011E). Cole et al. subsequently examined associations between these polymorphisms and serum calcium concentrations in 163 healthy women and demonstrated that the A986S polymorphism is one