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G Proteins — The Disease Spectrum Expands

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G proteins (guanine nucleotide-binding proteins) are heterotrimers composed of guanosine triphosphate-binding alpha subunits and tightly linked beta and gamma subunits. They couple a vast array of receptors (G-protein-coupled receptors, the subject of the Nobel Prize in Chemistry this past year¹) to effectors that regulate diverse cellular processes. Of 15 human alpha-subunit genes, some, such as *GNAS*, are expressed ubiquitously; others are expressed only in specialized cells. G_s (the G protein encoded by *GNAS*) couples many hormone and neurotransmitter receptors to cyclic AMP stimulation, and it was the first G protein to be associated with human disease. Germline mutations that inactivate $G\alpha_s$ (the G-protein subunit α_s) were shown to cause the prototypical hormone-resistance disorder, pseudohypoparathyroidism.² Somatic activating mutations cause sporadic endocrine tumors and the McCune-Albright syndrome.² Mutations subsequently were identified in genes that encode dysfunctional $G\alpha$ proteins in rod photoreceptors in forms of night blindness and in cone photoreceptors in forms of color blindness, and mutations in *GNAL* have been linked to primary torsion dystonia.³

Somatic mutations that activate $G\alpha_q$ (the G-protein subunit α_q) and $G\alpha_{11}$ (the G-protein subunit α_{11}), closely related G proteins that activate intracellular ionized calcium-mediated signaling, have been associated with uveal melanoma.⁴ In this issue of the *Journal*, Nesbit⁵ and Mannstadt⁶ and their colleagues report that germline muta-

tions that inactivate $G\alpha_{11}$ cause hypercalcemic disorders and germline mutations that activate $G\alpha_{11}$ cause hypocalcemic disorders.

Calcium homeostasis is tightly regulated by parathyroid hormone. Parathyroid hormone secretion from the parathyroid glands is inhibited directly by increased serum levels of calcium. Primary hyperparathyroidism, the major cause of hypercalcemia in patients seen in an ambulatory setting, is caused by a neoplastic process in one or more parathyroid glands. This process leads to excess parathyroid hormone secretion, despite increased serum levels of calcium.

Familial hypocalciuric hypercalcemia is an autosomal dominant disease that, like primary hyperparathyroidism, is characterized by hypercalcemia and normal or elevated levels of serum parathyroid hormone.⁷ However, renal and skeletal manifestations of primary hyperparathyroidism are generally absent in patients with familial hypocalciuric hypercalcemia. Partial parathyroidectomy does not correct the hypercalcemia; thus, surgery is not indicated. The patient's family history and measurement of urinary calcium:creatinine ratios are key to distinguishing familial hypocalciuric hypercalcemia from primary hyperparathyroidism.

Previous studies have shown that many cases of familial hypocalciuric hypercalcemia are caused by heterozygous germline inactivating mutations in the gene encoding the calcium-sensing receptor (*CASR*). The calcium-sensing receptor is a G-protein-coupled receptor that is highly ex-

pressed in parathyroid and renal tubular cells, but it is also detectable in many other tissues.⁸ Calcium directly activates the calcium-sensing receptor, inhibiting parathyroid hormone secretion in the parathyroids and decreasing renal calcium reabsorption. Loss of one functional *CASR* allele in familial hypocalciuric hypercalcemia reduces the sensitivity of the parathyroid and renal cells to calcium; this leads to hypercalcemia and to hypocalciuria relative to the filtered load of calcium. Homozygous loss-of-function mutations in *CASR* cause severe, potentially lethal neonatal hyperparathyroidism that may necessitate total parathyroidectomy.⁸

Conversely, heterozygous germline activating mutations in *CASR* cause a form of autosomal dominant hypocalcemia and hypoparathyroidism.⁸ Such mutations cause increased parathyroid and renal sensitivity to calcium, so that parathyroid hormone is suppressed at low serum levels of calcium, often with hypercalciuria relative to the filtered load of calcium.

Although in some kindreds the gene causing familial hypocalciuric hypercalcemia has been linked to chromosome 3q13, the locus of *CASR*, linkage to distinct sites in other kindreds has indicated genetic heterogeneity. The type 3 variant of familial hypocalciuric hypercalcemia was found to be caused by missense mutations in *AP2S1*, the gene encoding the sigma 1 subunit of adaptor-related protein complex 2, a protein complex that is critical for clathrin-mediated endocytosis of the calcium-sensing receptor.⁹

Now, Nesbit and colleagues have identified heterozygous germline inactivating mutations in *GNA11*, the gene encoding the alpha subunit of G11, in affected members of a kindred with another form of familial hypocalciuric hypercalcemia, type 2, and in one unrelated patient.⁵ *GNA11* localizes to chromosome 19p13.3, the linkage site for familial hypocalciuric hypercalcemia type 2.

Nesbit and colleagues⁵ and Mannstadt and colleagues also identified four different heterozygous missense mutations in *GNA11* in patients with autosomal dominant hypocalcemia in whom *CASR* mutations had not been detected. The findings of three-dimensional modeling of $G\alpha_{11}$ and heterologous expression studies indicate that the *GNA11* mutations identified are probably functionally important. Since G11 mediates signaling by the calcium-sensing receptor, *GNA11*-inactivating mutations would reduce and activating mutations would increase the sensitivity of

the parathyroid to calcium. Reduced sensitivity would lead to familial hypocalciuric hypercalcemia, and increased sensitivity would lead to autosomal dominant hypocalcemia.

The present findings expand the spectrum of diseases caused by mutant G protein, but they also raise interesting questions. Gq and G11 are considered to be functionally redundant and overlap in expression. Why, then, should loss of a single *GNA11* allele cause familial hypocalciuric hypercalcemia? In mice, homozygous knockout of *GNA11* does not cause major hypercalcemia, but homozygous knockout of both *GNAQ* and *GNA11* in the parathyroid glands causes severe neonatal hyperparathyroidism.¹⁰ Nesbit and colleagues⁵ suggest that high expression of $G\alpha_{11}$ relative to $G\alpha_q$ in human parathyroids explains the difference in phenotype.

Also, in the present study by Nesbit et al., what caused familial hypocalciuric hypercalcemia in patients in whom mutations in *CASR*, *GNA11*, and *AP2S1* were not detected? Finally, why should germline mutations (either activating or inactivating) in *GNA11*, a gene expressed in many tissues, lead only to impaired calcium homeostasis? Understanding the relationship between genotype and phenotype continues to be a major challenge in the genomic era.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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