of a meta-analysis of studies of the effect of reduced dietary salt on the incidence of cardiovascular events and death.[1] The authors of the Cochrane report wrote that there was "no strong evidence of benefit." In a summary statement, we wrote that this particular Cochrane analysis concluded that reducing dietary salt intake did not decrease the risk of death or cardiovascular disease. Stigler et al. suggest that "indeterminate results," rather than no significant effect, would be a more appropriate interpretation of the analysis. Both interpretations may be correct. Although it may not be possible to reject the null hypothesis with certainty (i.e., no effect of reduced salt), the analysis should have been powered to detect a clinically meaningful difference, and the conclusion of no effect provides more guidance than "indeterminate results" to clinical decision makers. Focusing on these subtleties, however, misses the point we made regarding the Cochrane report. As analyzed by others[2] and as cited in our review, after the exclusion of a trial involving patients with heart failure who received aggressive diuretic therapy and combining data for patients with and without hypertension, reduced salt intake was shown to be associated with a significant reduction in the rate of cardiovascular events.

Thornton raises important questions: do recommended reductions of dietary sodium stimulate the renin–angiotensin–aldosterone axis, and does this in turn contribute to cardiovascular disease? In trials of abrupt and severe salt restriction, plasma renin activity and serum aldosterone levels were increased. Although long-term, modest reductions in salt intake result in small, physiologic increases in plasma renin activity,[3] the preponderance of evidence suggests that a reduced salt intake is associated with a decreased risk of cardiovascular events and death. Furthermore, it is worth remembering that diuretics remain one of the most effective antihypertensive therapies, and their beneficial effect on cardiovascular disease is well documented.[4] Nevertheless, as we suggested, in terms of safety, the lower limit of salt consumption has not been clearly defined.

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Since publication of their article, the authors report no further potential conflict of interest.

1. Taylor RS, Ashton KE, Moxham T, Hooper L, Ebrahim S. Reduced dietary salt for the prevention of cardiovascular disease: a meta-analysis of randomized controlled trials (Cochrane review). Am J Hypertens 2011;24:843-53.
2. He FJ, MacGregor GA. Salt reduction lowers cardiovascular risk: meta-analysis of outcome trials. Lancet 2011;378:380-2.
3. He FJ, Li J, Macgregor GA. Effect of longer term modest salt reduction on blood pressure: Cochrane systematic review and meta-analysis of randomised trials. BMJ 2013;346:f1325.
4. Chobanian AV, Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560-72. [Erratum, JAMA 2003;290:197.]

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Germline Mutations Affecting Gα_{11} in Hypoparathyroidism

TO THE EDITOR: Extracellular calcium levels are tightly regulated by parathyroid hormone (PTH). Insufficient production of this hormone, as observed in nonsyndromic isolated hypoparathyroidism, can be caused by mutations in *PTH* or the genes encoding the parathyroid-specific transcription factor glial cells missing 2 (*GCM2*) or the calcium-sensing receptor (*CaSR*). However, most cases of isolated hypoparathyroidism remain genetically undefined.[1]

We investigated two unrelated white families in which 15 living members had clinical and laboratory findings consistent with autosomal dominant isolated hypoparathyroidism (Fig. 1). After ruling out the presence of mutations in *CaSR*, *PTH*, and *GCM2* in the index cases (not shown), a genomewide scan revealed linkage to a single chromosomal region for Family A (19p13.3; LOD score, 3.0). Candidate gene sequencing resulted in the identification of a heterozygous nucleotide change in exon 2 of *GNA11* (c.178C→T; p.Arg60Cys), the gene encoding the α subunit of the guanine nucleotide-binding protein G_{11} (Gα_{11}) (Fig. S1 in the Supplementary

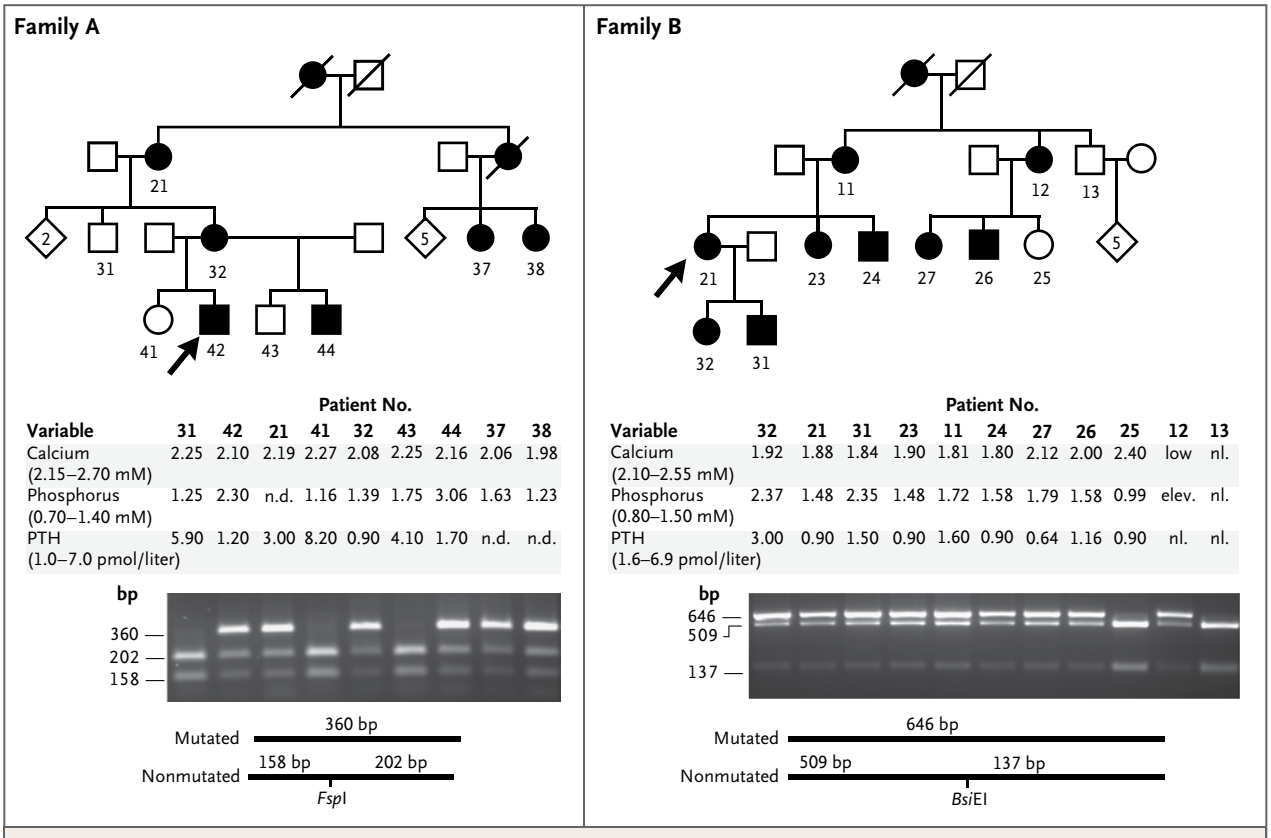

Figure 1. Pedigrees and Laboratory and Genetic Findings for Two Families with Autosomal Dominant Isolated Hypoparathyroidism.

Squares indicate male family members, circles female family members, black symbols affected family members, and white symbols unaffected family members; slashes indicate deceased family members; numbers outside squares and circles indicate family members for whom DNA was available for testing; numbers within white rhombi indicate the number of unaffected siblings. Arrows point to the index cases. Measurements for calcium, phosphorus, and parathyroid hormone (PTH) are shown (with adult normal ranges in parentheses) and reflect values at presentation except in the case of Patients 21 and 32, from whom samples were drawn after treatment was initiated. For affected members, mean (±SE) pretreatment serum levels of calcium were 2.15±0.02 mmol per liter in Family A and 1.91±0.04 mmol per liter in Family B, mean levels of phosphorus were 2.06±0.40 and 1.79±0.13 mmol per liter, respectively, and mean levels of PTH were 1.97±0.42 and 1.33±0.27 pmol per liter, respectively. Values for other variables among patients with autosomal dominant isolated hypoparathyroidism for whom mean pretreatment levels were available were as follows: magnesium, 0.77±0.02 mmol per liter (10 patients; normal range, 0.7 to 1.0); 1,25-dihydroxyvitamin D, 81.8±10.0 pmol per liter (8 patients; normal range, 40 to 150); and urinary calcium:creatinine ratio, normal when measured (8 patients; normal range, <0.6 mmol:mmol). Two novel mutations affecting $G\alpha_{11}$ (R60C and S211W) were identified by means of genetic linkage analysis for Family A and exome sequencing of a DNA sample from two affected members of Families A and B (see Fig. 1 in the Supplementary Appendix). Digestion of DNA (amplified by means of polymerase chain reaction) with the endonucleases FspI and BsiEI, respectively, confirmed the two mutations and revealed their segregation with the disease.

Appendix, available with the full text of this letter at NEJM.org). Whole-exome sequencing of two affected members of Family A (Patients 37 and 44) confirmed this nucleotide transition. Exome sequencing of two members of Family B (Patients 26 and 31) revealed a heterozygous nucleotide transversion in exon 5 of *GNA11* (c.632C→G; p.Ser211Trp); no additional variant that affects the same gene in both families was identified. The nucleotide changes were present only in affected family members, and both changes affect amino acid residues that are highly conserved in $G\alpha_{11}$ and the closely related $G\alpha_q$.

$G\alpha_{11}$ and $G\alpha_q$ mediate the intracellular signaling that depends on the generation of inositol 1,4,5-trisphosphate and the activation of protein kinase C and occurs downstream of CaSR,[2] the

main regulator of PTH synthesis and secretion. Homozygous inactivating *CaSR* mutations cause severe neonatal hyperparathyroidism, as does the combined parathyroid-specific ablation of $G\alpha_{11}$ and $G\alpha_q$ in mice.[3,4] Conversely, activating *CaSR* mutations lead to hypocalcemia because of inappropriate PTH secretion.[1] The latter findings are similar to those reported for our families with autosomal dominant isolated hypoparathyroidism, thus making it plausible to suggest that the identified $G\alpha_{11}$ mutants increase signaling at this receptor.

To evaluate this hypothesis, we analyzed both mutants with the use of molecular modeling (Fig. S2 in the Supplementary Appendix). On the basis of the proposed crystal structure of $G\alpha_{11}$, it is probable that the replacement of arginine 60 with cysteine (R60C mutant) in helix α1 of the guanosine triphosphatase (GTPase) domain will disrupt the intramolecular hydrogen bond with asparagine 71, located in αA of the helical domain. This mutant is therefore predicted to destabilize the "closed clamshell" conformation of the helical and GTPase domains, thus allowing either a faster exchange of guanosine diphosphate with GTP or disrupting $G\alpha_{11}$ contacts with regulatory proteins. In contrast, the replacement of serine 211 in the switch II region of $G\alpha_{11}$ with tryptophan (S211W mutant) is predicted to disrupt the binding of the mutant α subunit to the β subunit, thereby enhancing agonist-dependent signaling.

Activating mutations affecting $G\alpha_{11}$ and $G\alpha_q$ cause uveal melanomas[5]; however, these genetic changes are somatic, as are most disease-causing mutations in other G proteins (Table S1 in the Supplementary Appendix). In fact, only a few activating germline mutations affecting G proteins appear to be compatible with life; these include maternal mutations affecting $G\alpha_s$ that cause gonadotropin-independent male precocious puberty or neonatal diarrhea in combination with pseudohypoparathyroidism type 1a, and three murine germline mutations affecting $G\alpha_q$ or $G\alpha_{11}$ that lead to dermal hyperpigmentation. The inherited mutations affecting $G\alpha_{11}$ in family members with autosomal dominant isolated hypoparathyroidism are therefore remarkable, particularly since obvious abnormalities affect only the regulation of mineral-ion homeostasis. In summary, genomewide linkage analysis, combined with whole-exome sequencing, revealed two different heterozygous mutations affecting $G\alpha_{11}$ as novel causes of autosomal dominant isolated hypoparathyroidism.

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

1. Shoback D. Hypoparathyroidism. N Engl J Med 2008;359:391-403.
2. Hofer AM, Brown EM. Extracellular calcium sensing and signalling. Nat Rev Mol Cell Biol 2003;4:530-8.
3. Ho C, Conner DA, Pollak M, et al. A mouse model for familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Nat Genet 1995;11:389-94.
4. Wettschureck N, Lee E, Libutti SK, Offermanns S, Robey PG, Spiegel AM. Parathyroid-specific double knockout of Gq and G11 alpha-subunits leads to a phenotype resembling germline knockout of the extracellular Ca2+-sensing receptor. Mol Endocrinol 2007;21:274-80.
5. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in *GNA11* in uveal melanoma. N Engl J Med 2010;363:2191-9.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Mannstadt M, Harris M, Bravenboer B, et al. Germline mutations affecting Gα_{11} in hypoparathyroidism. N Engl J Med 2013;368:2532-4. DOI: 10.1056/NEJMc1300278

Supplementary Appendix

Germline Mutations Affecting $G\alpha_{11}$ in Hypoparathyroidism

Table of Contents

Supplemental description of the index cases and their families

Two Caucasian families with autosomal dominant isolated hypoparathyroidism were studied (see Fig. 1). The male index case of family A (subject 42, arrow in Fig. 1, left panel) was diagnosed at the age of 2 years with type 1 diabetes mellitus; at that time, total calcium level was within normal limits. At the age of 5 years he presented with generalized seizures, some of which were not associated with hypoglycemia; carbamazepine was given for 12 months and seizures did not re-occur after discontinuing this medication. At age 14 years, he complained of tremulousness and muscle cramps, and was found to be hypocalcemic with inappropriately low PTH levels. Review of the family history at that time revealed autosomal dominant transmission of hypocalcemia on his maternal side.

In family B, the index case (subject 21, arrow in Fig. 1, right panel) was diagnosed with isolated hypoparathyroidism when she presented with chronic fatigue and occasional muscle cramps at age 20; her laboratory evaluation revealed mild hypocalcemia and mild hyperphosphatemia with a low PTH level. Nine other family members were also diagnosed with isolated hypoparathyroidism; all had similarly mild symptoms of hypocalcemia.

None of the affected members in either family had a history of muco-cutaneous candidiasis, hearing loss, or renal abnormalities, and clinical examinations were unremarkable; in particular there was no evidence for skin changes.

Supplemental Methods

Consents and DNA collection

After obtaining written informed consent through our IRB approved protocol, blood samples were collected from affected and unaffected members of both families for DNA extraction using established methods.

Genetic Analysis

Sequence analysis of *CASR* was performed through the institutions of M.H. and B.B., respectively; *PTH* and *GCMB* were sequenced as described [1]. DNA samples from family A (3 healthy and 6 living affected) were genotyped using the InfiniumLinkage-24 SNP chip and multipoint linkage analysis was performed using GeneHunter [2,3]. For whole-exome sequencing, libraries were constructed with DNA from two affected members of each family (subjects 37 and

44 for family A; subjects 26 and 31 for family B) using the Agilent SureSelect Human All Exon Kit v2 [4] followed by massively parallel sequencing using an Illumina HiSeq Sequencer. Sequence data processing and variant calling was done using GATK [5] and annotation was performed using snpEff [6]; variants were considered to be potentially disease-causing when the allele frequency was ≤0.1% in 5,400 European control samples from the NHLBI Exome Sequencing Project (ESP), in dbSNP, and in the 1000 Genomes Project. PolyPhen2 was used to predict probably damaging rare missense, nonsense, or essential splice site variants. Identified mutations were confirmed by Sanger sequencing and restriction enzymatic digestion of PCR-amplified genomic DNA using the endonucleases Fsp1 and BsiEI, respectively.

Structural Analysis

Structural analyses and predictions are based on the crystal structure of a soluble, fully functional rat $G\alpha_{i/q}$ chimera, in which the N-terminal helix was replaced with that of mouse $G\alpha_{i1,}$ in complex with bovine $G\beta1/G\gamma2$ and the inhibitor YM-254890 (PDB ID 3AH8) [7]. Superposition with other heterotrimeric G protein complexes indicated that the chimeric substitution and inhibitor do not significantly alter the tertiary structure in the vicinity of the mutations. The mutations were modeled in the most common rotomer conformation compatible with the $G\alpha_{i/q}$ structure. Structural figures rendered with PyMOL [8] (DeLano Scientific LLC, Palo Alto, CA).

Table S1:
Diseases caused by somatic or germline mutations of guanine nucleotide-binding proteins

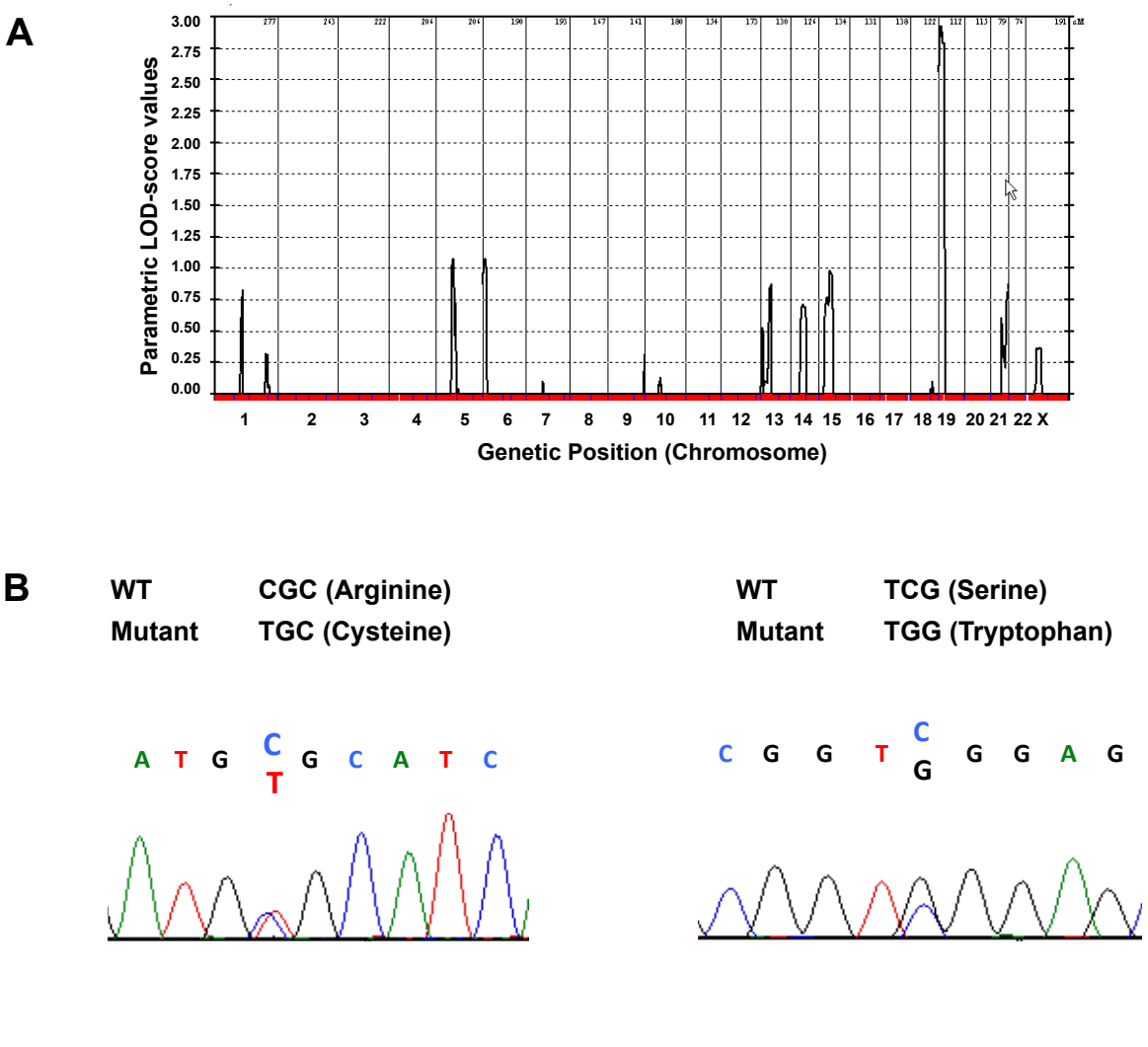
G protein	Mode of action	Disorders caused by somatic mutations	Mutation	Ref	Disorders caused by germline mutations	Mutation	Ref
$G\alpha_s$	activating	Pituitary adenomas, McCune-Albright Syndrome, and Fibrous Dysplasia	Arg^{201} Gln^{227}	9			
	activating/ LoF				Testotoxicosis/ Pseudohypoparathyroidism Neonatal diarrhea/ Pseudohypoparathyroidism	Ser^{366} $AVDT^{366-369}$ repeat	10 11
	inactivating or Δ methylation				Pseudohypoparathyroidism 1a or 1b	Multiple	12
$G\alpha_{t1}$	inactivating				Blindness (Nougaret)	Asp^{38}	13
$G\alpha_{t2}$	inactivating				Achromatopsia	Multiple	14
G_i	activating	Adrenocortical and ovarian tumors	Arg^{179}	9			
$G\alpha_q$	activating	Uveal Melanomas	Arg^{183} Gln^{209}	15	*Mouse dark skin**	Met^{179} Leu^{335}	16
$G\alpha_{11}$	activating	Uveal Melanomas	Arg^{183} Gln^{209}	17	*Mouse dark skin**	Ile^{63}	16
$G\alpha_{olf}$	inactivating				Primary Torsion Dystonia	Multiple	18

LoF; loss of function; *obtained through chemical mutagenesis in mice

Supplemental Figure 1:

Panel A: A genome-wide linkage scan for family A using all available members revealed a single linked region comprising approximately 10 Mb on chromosome 19p13.3 flanked by markers rs731714 and rs280521 (LOD score 3.0). Chromosomal location on the x-axis and parametric LOD score values on the y-axis.

Panel B: Nucleotide sequence analysis of *GNA11* exon 2 revealed a heterozygous nucleotide change, c.178C>T (p.Arg60Cys) for the index case 42 in family A (left panel). Nucleotide sequence analysis of *GNA11* exon 5 revealed a heterozygous nucleotide change, c.632C>G (p.Ser211Trp) for the index case 21 in family B (right panel).

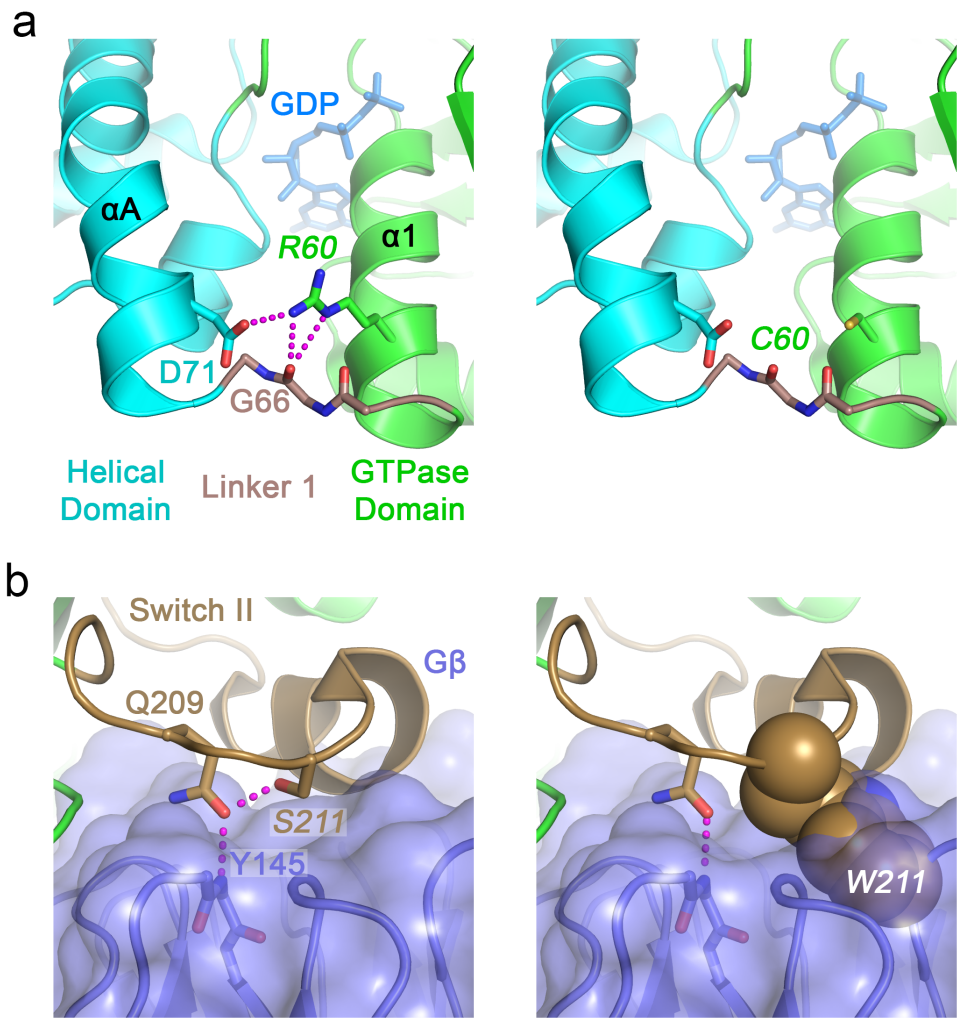


Supplemental Figure 2: Structural Analysis of Mutants

Model of $G\alpha_{11}$ based on the crystal structure of the $G\alpha_{i/q}\beta\gamma$ heterotrimeric complex (PDB ID 3AH8) [7]. Ribbon rendering shows the GTPase domain (green) and the helix αA (cyan).

Panel A: GDP, Arg60 and other relevant residues are shown as stick models [19]. Note polar interactions (magenta dashes) of Arg60 with Asp71 and the main chain carbonyl of Gly66, which likely stabilize the interaction of the helical with the GTPase domain. The polar interactions are disrupted by the cysteine substitution (right).

Panel B: Interaction of Ser211 (stick model) with the β-subunit (blue cartoon with semitransparent surface) is shown on the left. Substitution of Ser211 with tryptophan (spheres) is predicted to interrupt the interaction with βγ subunits (right).



References

1. Mannstadt M, Bertrand G, Muresan M, et al. Dominant-negative GCMB mutations cause an autosomal dominant form of hypoparathyroidism. J Clin Endocrinol Metab 2008;93:3568-76.
2. Hoffmann K, Lindner TH. easyLINKAGE-Plus--automated linkage analyses using large-scale SNP data. Bioinformatics 2005;21:3565-7.
3. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 1996;58:1347-63.
4. Gnirke A, Melnikov A, Maguire J, et al. Solution hybrid selection with ultra-long oligonucleotides for massively parallel targeted sequencing. Nat Biotechnol 2009;27:182-9.
5. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2011;43:491-8.
6. Cingolani P, Platts A, Wang le L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly 2012;6:80-92.
7. Nishimura A, Kitano K, Takasaki J, et al. Structural basis for the specific inhibition of heterotrimeric Gq protein by a small molecule. Proc Natl Acad Sci U S A 2010;107:13666-71.
8. Ordog R. PyDeT, a PyMOL plug-in for visualizing geometric concepts around proteins. Bioinformation 2008;2:346-7.
9. Farfel Z, Bourne HR, Iiri T. The expanding spectrum of G protein diseases. N Engl J Med 1999;340:1012-20.
10. Iiri T, Herzmark P, Nakamoto JM, van Dop C, Bourne HR. Rapid GDP release from Gs alpha in patients with gain and loss of endocrine function. Nature 1994;371:164-8.
11. Makita N, Sato J, Rondard P, et al. Human Gs alpha mutant causes pseudohypoparathyroidism type Ia/neonatal diarrhea, a potential cell-specific role of the palmitoylation cycle. Proc Natl Acad Sci U S A 2007;104:17424-9.
12. Bastepe M, Jüppner H. Pseudohypoparathyroidism, Albright's hereditary osteodystrophy, and progressive osseous heteroplasia: disorders caused by inactivating GNAS Mutations. In: DeGroot L, Jameson J, eds. Endocrinology. 6th ed. Philadelphia: W. B. Saunders Co.; 2010.
13. Dryja TP, Hahn LB, Reboul T, Arnaud B. Missense mutation in the gene encoding the alpha subunit of rod transducin in the Nougaret form of congenital stationary night blindness. Nat Genet 1996;13:358-60.
14. Kohl S, Baumann B, Rosenberg T, et al. Mutations in the cone photoreceptor G-protein alpha-subunit gene GNAT2 in patients with achromatopsia. Am J Hum Genet 2002;71:422-5.
15. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. Nature 2009;457:599-602.
16. Van Raamsdonk CD, Fitch KR, Fuchs H, de Angelis MH, Barsh GS. Effects of G-protein mutations on skin color. Nat Genet 2004;36:961-8.
17. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. N Engl J Med 2010;363:2191-9.
18. Fuchs T, Saunders-Pullman R, Masuho I, et al. Mutations in GNAL cause primary torsion dystonia. Nat Genet 2012;45:88-92.
19. Lambright DG, Noel JP, Hamm HE, Sigler PB. Structural determinants for activation of the alpha-subunit of a heterotrimeric G protein. Nature 1994;369:621-8.