of a meta-analysis of studies of the effect of reduced dietary salt on the incidence of cardiovascular events and death. 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 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, 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. Nevertheless, as we suggested, in terms of safety, the lower limit of salt consumption has not been clearly defined.

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Germline Mutations Affecting Ga11 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.

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 G11 (Ga11) (Fig. S1 in the Supplementary
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 Gα_{11} and the closely related Gα_q.

Gα_{11} and Gα_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
main regulator of PTH synthesis and secretion. Homozygous inactivating CaSR mutations cause severe neonatal hyperparathyroidism, as does the combined parathyroid-specific ablation of Gα11 and Gαq 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 Gα11 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 Gα11, 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 Gα11 contacts with regulatory proteins. In contrast, the replacement of serine 211 in the switch II region of Gα11 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 Gα11 and Gαq cause uveal melanomas5; 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 Gαs that cause gonadotropin-independent male precocious puberty or neonatal diarrhea in combination with pseudohypoparathyroidism type 1a, and three murine germline mutations affecting Gαq or Gα11 that lead to dermal hyperpigmentation. The inherited mutations affecting Gα11 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 Gα11 as novel causes of autosomal dominant isolated hypoparathyroidism.

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Supplementary Appendix

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

Supplementary Appendix

Germline Mutations Affecting Gα₁₁ in Hypoparathyroidism

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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
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 \(\leq 0.1\%\) 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 Ga\(_{i/q}\) chimera, in which the N-terminal helix was replaced with that of mouse Ga\(_{i1}\), in complex with bovine G\(\beta\)1/G\(\gamma\)2 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 Ga\(_{i/q}\) 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

<table>
<thead>
<tr>
<th>G protein</th>
<th>Mode of action</th>
<th>Disorders caused by somatic mutations</th>
<th>Mutation</th>
<th>Ref</th>
<th>Disorders caused by germline mutations</th>
<th>Mutation</th>
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<td>Gαs</td>
<td>activating</td>
<td>Pituitary adenomas, McCune-Albright Syndrome, and Fibrous Dysplasia</td>
<td>Arg(^{201}) Gln(^{227})</td>
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<td>activating/LoF</td>
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<td></td>
<td>Testotoxicosis/Pseudohypoparathyroidism Neonatal diarrhea/Pseudohypoparathyroidism</td>
<td>Ser(^{366}) AVDT(^{366-369}) repeat</td>
<td>10 11</td>
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<td>Pseudohypoparathyroidism 1a or 1b</td>
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<td>Multiple</td>
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<tr>
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<td>Asp(^{38})</td>
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<tr>
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<td>Gi</td>
<td>activating</td>
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<td>Arg(^{179})</td>
<td>9</td>
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<td>Uveal Melanomas</td>
<td>Arg(^{183}) Gln(^{209})</td>
<td>15</td>
<td>Mouse dark skin* Mouse dark skin*</td>
<td>Met(^{179}) Leu(^{335})</td>
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</tr>
<tr>
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<td>Uveal Melanomas</td>
<td>Arg(^{183}) Gln(^{209})</td>
<td>17</td>
<td>Mouse dark skin* Mouse dark skin*</td>
<td>Ile(^{53})</td>
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<tr>
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<td>Primary Torsion Dystonia</td>
<td>Multiple</td>
<td>18</td>
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</tr>
</tbody>
</table>

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 Gα₁₁ based on the crystal structure of the Gαᵢ/qβγ heterotrimeric complex (PDB ID 3AH8). 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. 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