ORIGINAL ARTICLE

Obesity-Associated *GNAS* Mutations and the Melanocortin Pathway

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ABSTRACT

BACKGROUND

GNAS encodes the $G\alpha_s$ (stimulatory G-protein alpha subunit) protein, which mediates G protein–coupled receptor (GPCR) signaling. *GNAS* mutations cause developmental delay, short stature, and skeletal abnormalities in a syndrome called Albright's hereditary osteodystrophy. Because of imprinting, mutations on the maternal allele also cause obesity and hormone resistance (pseudohypoparathyroidism).

METHODS

We performed exome sequencing and targeted resequencing in 2548 children who presented with severe obesity, and we unexpectedly identified 22 *GNAS* mutation carriers. We investigated whether the effect of *GNAS* mutations on melanocortin 4 receptor (MC4R) signaling explains the obesity and whether the variable clinical spectrum in patients might be explained by the results of molecular assays.

RESULTS

Almost all *GNAS* mutations impaired MC4R signaling. A total of 6 of 11 patients who were 12 to 18 years of age had reduced growth. In these patients, mutations disrupted growth hormone–releasing hormone receptor signaling, but growth was unaffected in carriers of mutations that did not affect this signaling pathway (mean standard-deviation score for height, −0.90 vs. 0.75, respectively; P=0.02). Only 1 of 10 patients who reached final height before or during the study had short stature. *GNAS* mutations that impaired thyrotropin receptor signaling were associated with developmental delay and with higher thyrotropin levels (mean [±SD], 8.4±4.7 mIU per liter) than those in 340 severely obese children who did not have *GNAS* mutations (3.9±2.6 mIU per liter; P=0.004).

CONCLUSIONS

Because pathogenic mutations may manifest with obesity alone, screening of children with severe obesity for *GNAS* deficiency may allow early diagnosis, improving clinical outcomes, and melanocortin agonists may aid in weight loss. *GNAS* mutations that are identified by means of unbiased genetic testing differentially affect GPCR signaling pathways that contribute to clinical heterogeneity. Monogenic diseases are clinically more variable than their classic descriptions suggest. (Funded by Wellcome and others.)

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This article was published on October 6, 2021, at NEJM.org.

N Engl J Med 2021;385:1581-92.
DOI: 10.1056/NEJMoa2103329
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Single-gene disorders that involve mendelian inheritance are individually rare, but collectively they account for 1 in 100 births and are a considerable burden for patients and families.[1] Although genetic testing has traditionally been informed by clinical characteristics alone, next-generation sequencing now permits the unbiased testing of multiple genes. However, this sequencing presents challenges, particularly when previously unreported rare variants are identified. In the absence of pathognomonic features, evidence of de novo inheritance, or both, rare variants are termed variants of uncertain significance.[2] The inability to link some of these variants to disease has major implications for clinical care. We evaluated patients who presented with severe obesity but were found to have mutations in a gene associated with a distinctive syndrome — Albright's hereditary osteodystrophy, or pseudohypoparathyroidism. We investigated potential explanations for the clinical variation we observed, using an approach that may inform the study of other monogenic diseases.

Albright's hereditary osteodystrophy is an autosomal dominant disorder that was first described by Fuller Albright in 1942.[3] Patients present with developmental delay, brachydactyly (shortened metacarpals, metatarsals, or distal phalanges, or all three) that is usually evident at 4 to 5 years of age, subcutaneous ossifications, childhood-onset obesity with short stature (i.e., final height that is 2 SD below the mean for sex [<3rd percentile]), and, in some cases, hormone resistance syndromes.[4-6] A clinical diagnosis is confirmed by the detection of heterozygous loss-of-function mutations in *GNAS*, the gene encoding Gα_s (stimulatory G-protein alpha subunit), which mediates signaling by hormones and ligands that bind to G protein–coupled receptors (GPCRs) to generate cyclic AMP.[7] Imprinting at the *GNAS* locus results in tissue-specific silencing of the paternally inherited *GNAS* allele.[8] In patients with mutations on maternally inherited alleles, which are preferentially expressed in the thyroid, pituitary, and renal proximal tubule, resistance develops to parathyroid hormone (PTH) and other hormones that signal through Gα_s-coupled receptors (pseudohypoparathyroidism type 1A), whereas patients with mutations on paternally inherited alleles have Albright's hereditary osteodystrophy without hormone resistance (pseudopseudohypoparathyroidism). Patients with pseudohypoparathyroidism type 1C have clinical features of type 1A, but in these patients, Gα_s bioactivity is normal in current assays.[9]

This study involved a subgroup of patients with severe obesity who were enrolled in the Genetics of Obesity Study (GOOS; www.goos.org.uk) and in whom mutations in known obesity genes had been ruled out. We performed exome sequencing and targeted resequencing, as previously described.[10,11] Unexpectedly, we found *GNAS* mutations in 22 patients in whom the diagnosis of pseudohypoparathyroidism had not been suspected on clinical grounds. We examined whether the effect of *GNAS* mutations on GPCR signaling might provide an explanation for the absence or presence of features of pseudohypoparathyroidism.

Methods

Study Patients and Ethical Approval

A total of 7000 patients in the United Kingdom with severe obesity (a body-mass index [BMI; the weight in kilograms divided by the square of the height in meters] standard-deviation score >3) of early onset (before 10 years of age) were enrolled in the GOOS cohort. Each patient (or the patient's parent if the patient was younger than 16 years of age) provided written informed consent; minors provided oral consent. Genetic studies were undertaken in 2548 children, as described previously.[10,11]

The study, which was approved by the multiregional ethics committee and the local research ethics committee of Cambridge, was conducted in accordance with the Declaration of Helsinki. The funding bodies had no role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; or the preparation, review, or approval of the manuscript or the decision to submit the manuscript for publication.

Clinical Measurements

GNAS mutation carriers were compared with severely obese children in the GOOS cohort in whom mutations in known obesity genes, including *GNAS*, had been ruled out. The standard-deviation score for height and BMI were calculated with the use of reference data for the United Kingdom.[12] Detailed methods are de-

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scribed in the Supplementary Appendix, available with the full text of this article at NEJM.org.

FUNCTIONAL CHARACTERIZATION OF *GNAS* MUTATIONS

$G\alpha_s$ mutants were generated by mutagenesis of the wild-type construct and transiently expressed in human embryonic kidney (HEK) 293 or *Gnas*$^{E2-/E2-}$ cells.[13] We measured cyclic AMP production (HitHunter cyclic AMP assay), and we used an enzyme complementation assay (NanoBiT, Promega) to measure the $G\alpha_s$–GPCR interaction. Details of these methods are described in the Supplementary Appendix.

STATISTICAL ANALYSIS

Results were analyzed with the use of GraphPad Prism 8 software. Anthropometric and biochemical data from *GNAS* mutation carriers were compared with data from other children in the GOOS cohort in whom mutations in known obesity genes, including *GNAS*, had been ruled out. These data were compared with the use of the two-tailed Mann–Whitney U test. For molecular data, the mean and standard error of the mean are reported.

RESULTS

GNAS MUTATIONS AND SEVERE OBESITY

We evaluated 22 patients who presented with severe childhood-onset obesity and among whom 19 heterozygous *GNAS* mutations were identified, including 16 missense, 2 nonsense, and 1 frameshift mutation (Fig. S1 in the Supplementary Appendix). The R265H mutation was present in 4 unrelated patients. Some mutations were reported previously in patients with pseudohypoparathyroidism (Table S1). Nine probands inherited mutations from their mothers, 8 of whom were overweight or obese (Table S2); 8 mutations arose de novo (Table 1). All the implicated variants, some of which were originally designated as variants of uncertain significance (and were found to be pathogenic in this study),[2] were submitted to the ClinVar repository under accession numbers SCV001573808 through SCV001573826.

None of the patients were thought to have pseudohypoparathyroidism when referred for evaluation of severe obesity. To determine whether the implicated variants affect the function of $G\alpha_s$ protein, we performed experiments in $G\alpha_s$-null cells. The standard assay of $G\alpha_s$ bioactivity cannot detect defective coupling of $G\alpha_s$ to GPCRs,[9] so we developed a protein–protein interaction assay to study this mechanism (Fig. 1A). We also measured receptor-dependent and receptor-independent cyclic AMP production and the interaction of $G\alpha_s$ with adenylyl cyclase 2 (Fig. 1A). We studied GPCRs that mediate hormone resistance and GPCRs involved in weight regulation. The nonsense and frameshift mutations resulted in proteins that were not expressed and caused a complete loss of function. All 16 missense mutant proteins were expressed at levels similar to those of normal (wild-type) $G\alpha_s$, but they had impaired coupling to GPCRs, cyclic AMP production, or both (Table S3).

GNAS MUTATIONS AND MC4R SIGNALING

We reviewed the clinical records of the patients and assessed features of pseudohypoparathyroidism that were present at the time of genetic diagnosis (Table 1 and Table S4). Although the mean (±SD) gestational age at birth of the *GNAS* mutation carriers was 39.6±1.7 weeks, the mean standard-deviation score for birth weight was low (−0.72), consistent with mild intrauterine growth restriction (Fig. S2). All the patients had accelerated weight gain in the first 6 months of life that led to severe obesity in childhood (Fig. 1B).

We investigated the effect of *GNAS* mutations on signaling by the $G\alpha_s$-coupled melanocortin 4 receptor (MC4R), which is critical to the regulation of appetite and weight and in which heterozygous loss-of-function mutations are the most common monogenic form of obesity.[14] Fourteen of 16 mutations impaired the interaction between $G\alpha_s$ and MC4R (Fig. 1C), MC4R-mediated cyclic AMP accumulation (Fig. 1D), MC4R-independent cyclic AMP accumulation, or all of these. The effect of *GNAS* mutations on MC4R signaling is sufficient to explain early-onset obesity in the patients (Table 1), many of whom were reported to have hyperphagia, a cardinal feature of MC4R deficiency.[14]

Fifteen of 16 *GNAS* mutations impaired coupling to or signaling by β_2- and β_3-adrenoreceptors (Fig. S3), which mediate thermogenesis in brown adipose tissue. These findings may explain hypothermia in infancy, bradycardia, constipation, urinary retention, bronchoconstriction, and the

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Table 1. Clinical Features of *GNAS* Mutation Carriers with Severe Obesity.*

Mutation	Sex	Age at Referral	Age at Genetic Diagnosis	Age at Onset of Obesity	Speech Delay or Learning Difficulties	Thyrotropin Level (Age)†	Age at Levothyroxine Treatment	Parathyroid Hormone Level (Age)‡	Brachydactyly or Ectopic Ossifications	Final Height§
			years			*mIU/liter*	*years*	*pg/ml*		*cm (percentile)*
c.124C→A (p.Arg42Ser)¶	M	1.2	12.0	0.3	Yes	4.8 (1.2 yr); 4.0 (13.2 yr); 4.6 (16.3 yr)	7	81 (13.2 yr)	Brachydactyly	165 (2nd–9th)
c.127C→G (p.Leu43Val)¶	F	2.8	4.8	0.1	No	6.3 (2.8 yr); 8.3 (5.3 yr); 9.4 (8.4 yr)	6–7, declined treatment	11 (2.8 yr); 48 (8.4 yr)	Brachydactyly	NA
c.489C→G (p.Tyr163*)‖	M	1.0	2.1	0.3	Yes	10.8 (1.0 yr); 13.3 (2.6 yr); 1.5 (7.1 yr)	2	151 (1.0 yr); 702 (2.6 yr); 451 (7.1 yr)	Ectopic ossifications	NA
c.507C→A (p.Tyr169*)‖	M	1.3	9.6	0.3	Yes	18.3 (1.3 yr); 7.7 (9.6 yr); 7.7 (11.6 yr)	2–8	75 (1.3 yr)	None	NA
c.563_566del (p.Asp189MetfsX14)**	M	1.2	10.3	0.2	Yes	0.5 (1.2 yr); 4.2 (10.6 yr)	2–10	283 (15.7 yr)	Brachydactyly	NA
c.595C→T (p.Arg199Cys)¶	M	2.6	9.2	0.3	Yes	3.7 (2.6 yr); 1.6 (10.1 yr); 2.9 (12.9 yr)	NA	18 (2.6 yr); 58 (10.1 yr); 47 (12.9 yr)	Brachydactyly	NA
c.682C→T (p.Arg228Cys)**	F	0.5	5.2	0.3	Yes	5.9 (0.5 yr); 4.2 (6.3 yr)	NA	213 (6.3 yr)	None	NA
c.692G→A (p.Arg231His)**	M	3.6	11.0	0.6	Yes	16.4 (14.3 yr)	<4	122 (14.3 yr)	Brachydactyly	NA
c.702G→C (p.Trp234Cys)¶	M	3.3	19.1	0.7	Yes	2.0 (19.5 yr); 3.1 (20.5 yr)	NA	57 (19.5 yr); 23 (20.5 yr)	None	175.6 (25th–50th)
c.773G→T (p.Arg258Leu)**	F	0.4	12.4	0.2	Yes	10.0 (0.4 yr); 10.1 (13.7 yr); 12.4 (16.3 yr)	3	96 (16.3 yr)	None	155.2 (9th)
c.772C→T (p.Arg258Trp)**	F	3.9	19.4	0.1	Yes	2.2 (3.9 yr); 6.4 (22.1 yr); 4.2 (23.4 yr)	3	149 (3.9 yr); 283 (22.1 yr); 141 (23.4 yr)	Brachydactyly	156 (9th)
c.794G→A (p.Arg265His) (1)**	F	13.9	19.4	0.4	Yes	NA	NA	NA	None	167 (75th)

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c.794G→A (p.Arg265His) (2)‖	F	11.0	19.2	0.5	Yes	4.3 (11.0 yr); 2.7 (23.8 yr); 1.9 (26.9 yr)	NA	1072 (26.9 yr)	None	160 (25th)
c.794G→A (p.Arg265His) (3)‖	F	2.8	8.0	0.8	Yes	NA	Unknown	NA	Brachydactyly	NA
c.794G→A (p.Arg265His) (4)**	F	0.9	5.2	0.1	Yes	5.0 (0.9 yr); 6.2 (5.7 yr)	NA	53 (0.9 yr); 155 (5.7 yr)	Brachydactyly	NA
c.917C→T (p.Ser306Leu)¶	F	7.1	16.8	0.3	Yes	2.2 (7.1 yr); 0.6 (17.5 yr); 1.0 (20.1 yr)	NA	40 (7.1 yr); 88 (17.5 yr)	Brachydactyly	153 (2nd)
c.1025G→A (p.Arg342Gln)¶	M	5.8	11.4	0.8	No	7.5 (5.8 yr); 3.3 (11.4 yr); 4.2 (16.3 yr)	NA	30 (5.8 yr); 58 (11.4 yr)	Brachydactyly	171.1 (25th)
c.1040G→C (p.Arg347Thr)¶	F	3.4	11.4	0.5	No	7.6 (3.4 yr); 3.5 (11.8 yr); 3.4 (13.4 yr)	2	17 (3.4 yr); 69 (11.8 yr); 60 (13.4 yr)	None	NA
c.1057G→A (p.Gly353Arg)‖	F	14.7	29.0	3.0	No	3.3 (14.7 yr)	NA	17 (14.7 yr)	NA	NA
c.1067G→A (p.Arg356His)¶	F	13.8	26.4	0.1	No	1.9 (13.8 yr); 0.4 (26.7 yr); 2.3 (28.3 yr)	NA	50 (26.7 yr); 22 (28.3 yr)	None	175 (98th)
c.1115T→C (p.Ile372Thr)**	F	3.6	3.2	0.8	Yes	Normal (7.6 yr)	NA	Normal (7.6 yr)	None	NA
c.1150C→G (p.Gln384Glu)¶	F	8.1	23.6	0.7	Yes	10.2 (8.1 yr); 9.3 (24.8 yr); 12.0 (26.9 yr)	12	56 (8.1 yr); 233 (26.9 yr)	None	157 (9th)

* The *GNAS* nucleotide coding transcript is National Center for Biotechnology Information reference sequence NM_000516.7, and the genomic coordinates are NC_000020.11:58839681–58911192 (*Homo sapiens* chromosome 20, build GRCh38.p13).NA denotes not available.
† The reference range for thyrotropin was 0.35 to 5.50 mIU per liter.
‡ The reference range for parathyroid hormone was 14 to 65 pg per milliliter.
§ The corresponding percentile for final height was based on United Kingdom reference data. Short stature is defined as a final height that is 2 SD below the mean for sex (<3rd percentile). A subset of variants (R42S, W234C, R258W, R258L, S306L, R342Q, and Q384E) was found to impair growth hormone–releasing hormone receptor signaling.
¶ This mutation was maternally inherited.
‖ The inheritance of this mutation was not known.
** This was a de novo mutation.

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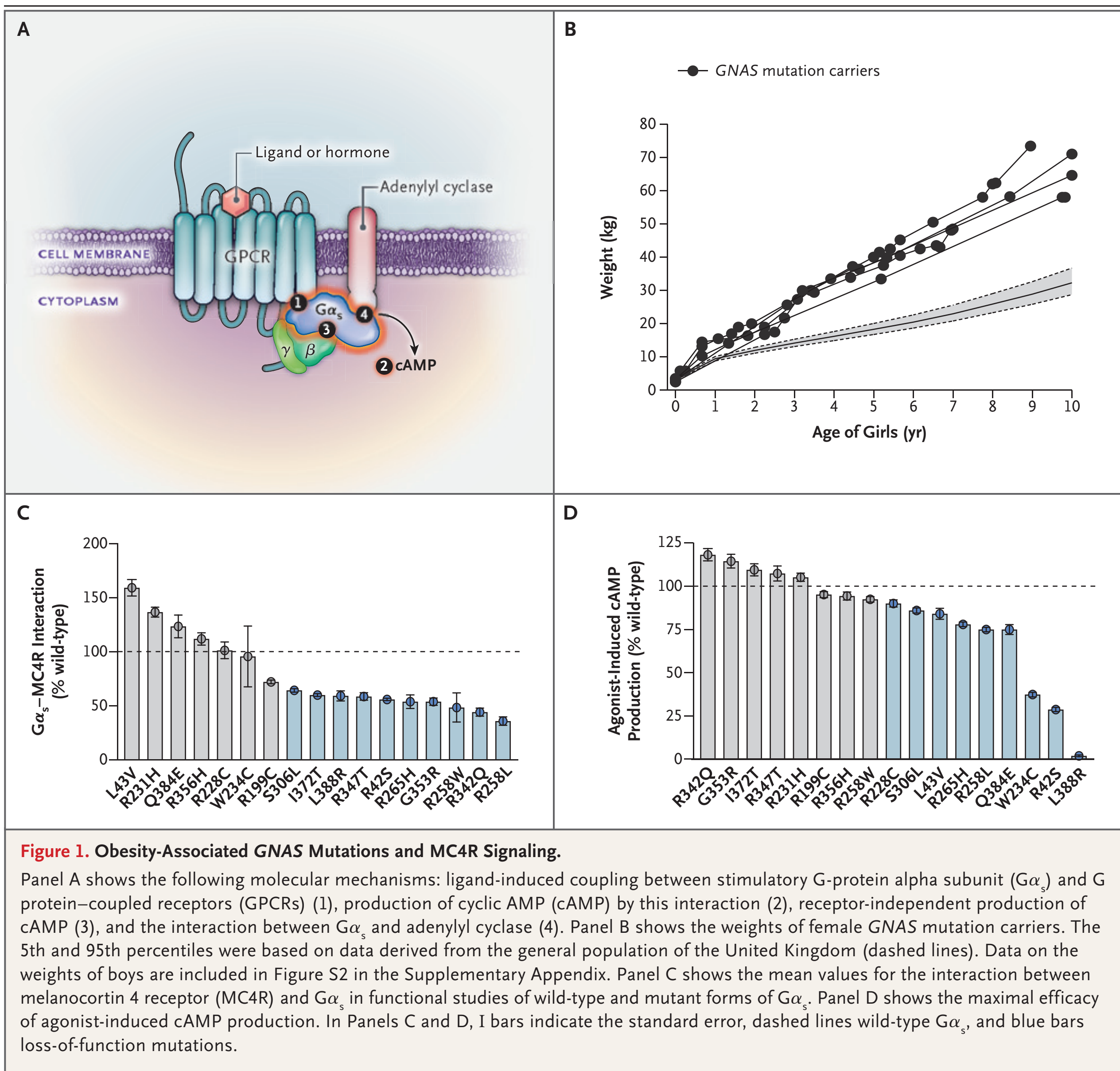

Figure 1. **Obesity-Associated *GNAS* Mutations and MC4R Signaling.**

Panel A shows the following molecular mechanisms: ligand-induced coupling between stimulatory G-protein alpha subunit ($G\alpha_s$) and G protein–coupled receptors (GPCRs) (1), production of cyclic AMP (cAMP) by this interaction (2), receptor-independent production of cAMP (3), and the interaction between $G\alpha_s$ and adenylyl cyclase (4). Panel B shows the weights of female *GNAS* mutation carriers. The 5th and 95th percentiles were based on data derived from the general population of the United Kingdom (dashed lines). Data on the weights of boys are included in Figure S2 in the Supplementary Appendix. Panel C shows the mean values for the interaction between melanocortin 4 receptor (MC4R) and $G\alpha_s$ in functional studies of wild-type and mutant forms of $G\alpha_s$. Panel D shows the maximal efficacy of agonist-induced cAMP production. In Panels C and D, I bars indicate the standard error, dashed lines wild-type $G\alpha_s$, and blue bars loss-of-function mutations.

reduced lipolytic response to epinephrine.[15] Fasting insulin and glucose levels in carriers of *GNAS* mutations were similar to those seen in obese children in the GOOS cohort (Fig. S4).

GNAS MUTATIONS, GHRH RECEPTOR SIGNALING, AND GROWTH

Pseudohypoparathyroidism is associated with reduced growth, in part because of growth hormone deficiency caused by resistance at the level of the growth hormone–releasing hormone (GHRH) receptor.[16,17] In addition, $G\alpha_s$ mediates the effects of PTH and PTH-related protein on chondrocytes in the growth plate.[18,19]

We plotted height data for 2270 boys and 2529 girls with severe obesity who were enrolled in GOOS. As expected, these children had a faster growth trajectory in early childhood than that indicated by the reference values derived from the general population of the United Kingdom (Fig. 2A and Fig. S5). Children with *GNAS* mutations had accelerated growth before 12

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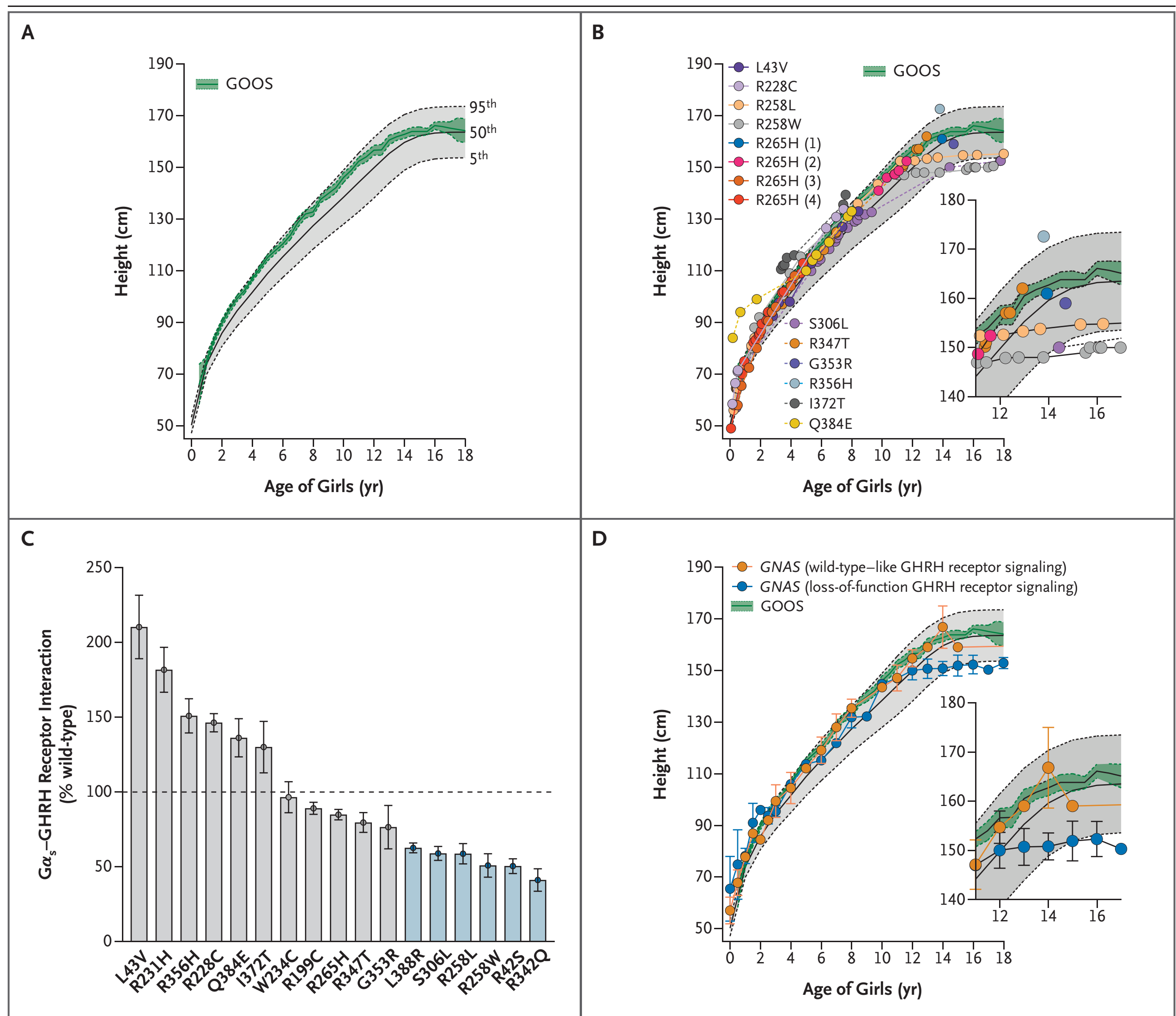

Figure 2. Impaired GHRH Receptor Signaling and Reduced Growth.

Panels A and B show the heights of 2529 severely obese girls who were enrolled in the Genetics of Obesity Study (GOOS) and female *GNAS* mutation carriers, respectively; comparable data on boys are included in Figure S5 in the Supplementary Appendix. In Panels A, B, and D, dashed lines indicate the 5th and 95th percentiles according to data derived from the general population of the United Kingdom. In Panels B and D, the insets show the same data on an expanded y axis. Panel C shows mean values for the interaction between the growth hormone–releasing hormone (GHRH) receptor and $G\alpha_s$ in functional studies of wild-type and mutant forms of $G\alpha_s$. The dashed line indicates wild-type $G\alpha_s$, blue bars loss-of-function mutations, and I bars the standard error. Panel D shows the height for female *GNAS* mutation carriers and the functional effects of their mutations on GHRH receptor signaling. I bars indicate the standard error.

years of age, with a mean standard-deviation score for height of 0.92; only 1 patient (with the Y163X mutation) had short stature at enrollment. In 14 of 18 children with *GNAS* mutations, growth trajectories before 12 years of age were similar to those in other severely obese children (Fig. 2B); bone age was generally not advanced (Table S5). However, 6 of 11 *GNAS* mutation carriers had a reduced pubertal growth spurt, reduced final height, or both (Fig. 2B and Table 1). One patient (with the S306L mutation) of 10 patients who attained final height before or during the study had short stature (Table 1).

Patients with a reduced pubertal growth

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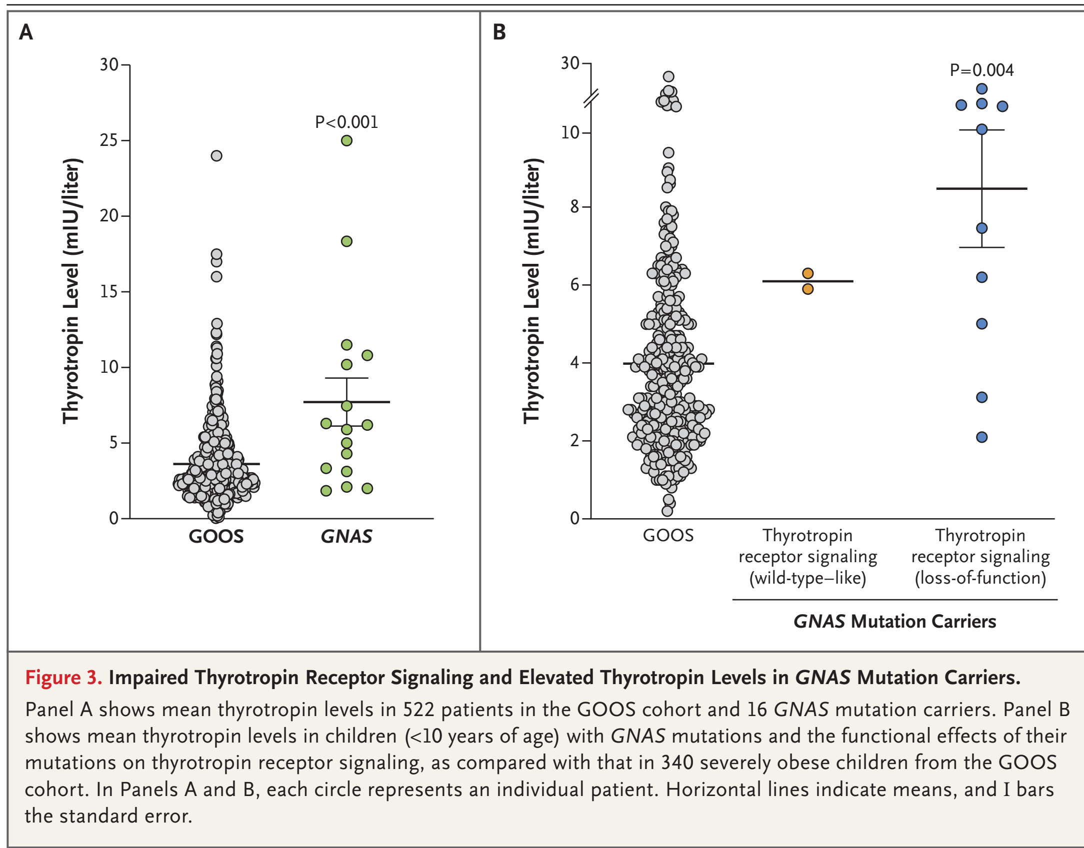

Figure 3. Impaired Thyrotropin Receptor Signaling and Elevated Thyrotropin Levels in *GNAS* Mutation Carriers.

Panel A shows mean thyrotropin levels in 522 patients in the GOOS cohort and 16 *GNAS* mutation carriers. Panel B shows mean thyrotropin levels in children (<10 years of age) with *GNAS* mutations and the functional effects of their mutations on thyrotropin receptor signaling, as compared with that in 340 severely obese children from the GOOS cohort. In Panels A and B, each circle represents an individual patient. Horizontal lines indicate means, and I bars the standard error.

spurt carried *GNAS* mutations that impaired GHRH receptor signaling (mean standard-deviation score for height, −0.90) (Fig. 2C and 2D), whereas growth followed predicted percentiles in those with *GNAS* mutations that did not impair function (mean standard-deviation score for height, 0.75; P=0.02). Most *GNAS* mutations impaired luteinizing hormone–chorionic gonadotropin receptor signaling and follicle-stimulating hormone receptor signaling. All the patients had pubertal development, which suggested sufficient activity in the residual hypothalamo–pituitary–gonadal axis. A reduced pubertal growth spurt in some patients may indicate growth hormone deficiency, the consequences of impaired PTH or PTH-related protein signaling on the growth plate, or both. Since MC4R deficiency is associated with accelerated growth (because of hyperinsulinemia),[20] impaired MC4R signaling resulting from *GNAS* mutations may counterbalance the negative effects of partial growth hormone deficiency on growth in early childhood.

GNAS MUTATIONS, THYROTROPIN RECEPTOR SIGNALING, AND DEVELOPMENTAL DELAY

GNAS mutations can affect signaling by the $G\alpha_s$-coupled thyrotropin receptor,[21,22] which drives thyroid hormone synthesis. Thyrotropin resistance (a high thyrotropin level with normal or low free thyroxine [free T_4] levels in the absence of goiter and antithyroid antibodies[17]) was evident in 11 of 16 *GNAS* mutation carriers (Table 1). In some instances, an elevated thyrotropin level was attributed to subclinical hypothyroidism, which is a frequent finding in childhood obesity.[23] Although the mean thyrotropin level was within the normal range in 522 severely obese children in the GOOS cohort, this level was elevated in 16 *GNAS* mutation carriers (Fig. 3A) (P<0.001). Free T_4 levels were normal in children with *GNAS* deficiency, a finding that is consistent with compensation by the thyroid axis (Fig. S6). Carriers of loss-of-function *GNAS* mutations had significantly higher mean (±SD) thyrotropin levels than 340 severely obese children from the GOOS cohort (8.4±4.7 vs. 3.9±2.6 mIU per liter;

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P=0.004) (Fig. 3B), whereas carriers of mutations with wild-type–like activity had similar thyrotropin levels.

All 17 of the *GNAS* mutations that were associated with developmental delay impaired thyrotropin receptor–mediated cyclic AMP production, $G\alpha_s$–thyrotropin receptor interaction, or both. Some patients received levothyroxine (Table 1); however, because thyrotropin resistance was not recognized, treatment was often discontinued. Partial thyrotropin resistance may explain the low basal metabolic rate reported previously in patients with *GNAS* deficiency[24] and in 2 of 6 patients in our study; in 1 patient with the R265H mutation, the measured basal metabolic rate was 60% of that predicted according to age, sex, and body composition, and in 1 patient with the W234C mutation, this rate was 82% of that predicted.

POOR CORRELATION BETWEEN SKELETAL ABNORMALITIES AND PTH RESISTANCE

GNAS mutations that affect signaling by the $G\alpha_s$-coupled PTH receptor lead to PTH resistance, reduced 1-alpha hydroxylation of vitamin D despite hypocalcemia, and impaired PTH-stimulated down-regulation of phosphate cotransporters, which reduce urinary phosphate excretion and cause hyperphosphatemia. PTH resistance in the proximal renal tubules can be compensated for by the actions of PTH in bone and the thick ascending renal tubule that can be mediated by G proteins other than $G\alpha_s$,[25] and thus it is not affected by *GNAS* mutations. As such, an elevated PTH level can maintain normocalcemia for some time. In our cohort, 11 patients had an elevated PTH level with normal calcium levels in early childhood (Table 1).

The majority of obesity-associated *GNAS* mutations impaired PTH type 1 receptor signaling. In keeping with the paucity of truncating mutations,[26] only one patient (with the Y163X mutation) had subcutaneous ossifications. Ten patients had brachydactyly, which was often subtle (Fig. 4A and Table 1). These findings indicate that the skeletal features of pseudohypoparathyroidism do not directly correlate with the degree of PTH resistance (Fig. 4A through 4C) and may be absent in some patients with *GNAS* mutations that nonetheless impair signaling by the PTH receptor (Table 1 and Fig. S7).

DISCUSSION

Unbiased genetic screening of a large cohort of patients who presented with severe childhood-onset obesity revealed an unexpectedly high prevalence of loss-of-function *GNAS* mutations. *GNAS* sequencing has traditionally been performed only in patients with the classic features of pseudohypoparathyroidism, so our findings suggest that the true prevalence of pathogenic mutations is much higher than previously considered.

We found that 75% of *GNAS* missense mutations disrupted the interaction between $G\alpha_s$ and GPCRs. Although caution is warranted when interpreting specific assay values because of limited power and the effects of multiple testing, these assays provide an additional readout of function, which may improve the diagnostic yield in patients with variants of uncertain significance in *GNAS* and in those with pseudohypoparathyroidism type 1C. As also suggested by Grüters-Kieslich and colleagues,[27] we conclude that screening for mutations in *GNAS* should be incorporated into the diagnostic workup for severe childhood-onset obesity. In view of our findings, the diagnostic approach to patients with clinical features of pseudohypoparathyroidism[28,29] should also be applied to the evaluation of other patients with severe childhood-onset obesity. Early diagnosis of *GNAS* deficiency guides monitoring for hormone resistance, recognition of hypocalcemia as a cause of seizures, and treatment with levothyroxine when appropriate. Growth velocity and pubertal development should be monitored so that growth hormone therapy can be initiated before fusion of the growth plate.

Brain-specific deletion of the maternal $G\alpha_s$ allele impairs the ability of an MC4R agonist to reduce weight in mice; this suggests that centrally expressed MC4Rs mediate obesity in *GNAS* deficiency.[30] We found that obesity-associated *GNAS* mutations in humans impair MC4R signaling. Impaired MC4R signaling leads to hyperphagia, impaired sympathetic nervous system activation, accelerated growth, and weight gain.[14,31] Even partial loss-of-function heterozygous *MC4R* mutations cause severe obesity,[14,32] so these findings provide an explanation for why severe obesity develops in patients with missense mutations in *GNAS* before other features of classic

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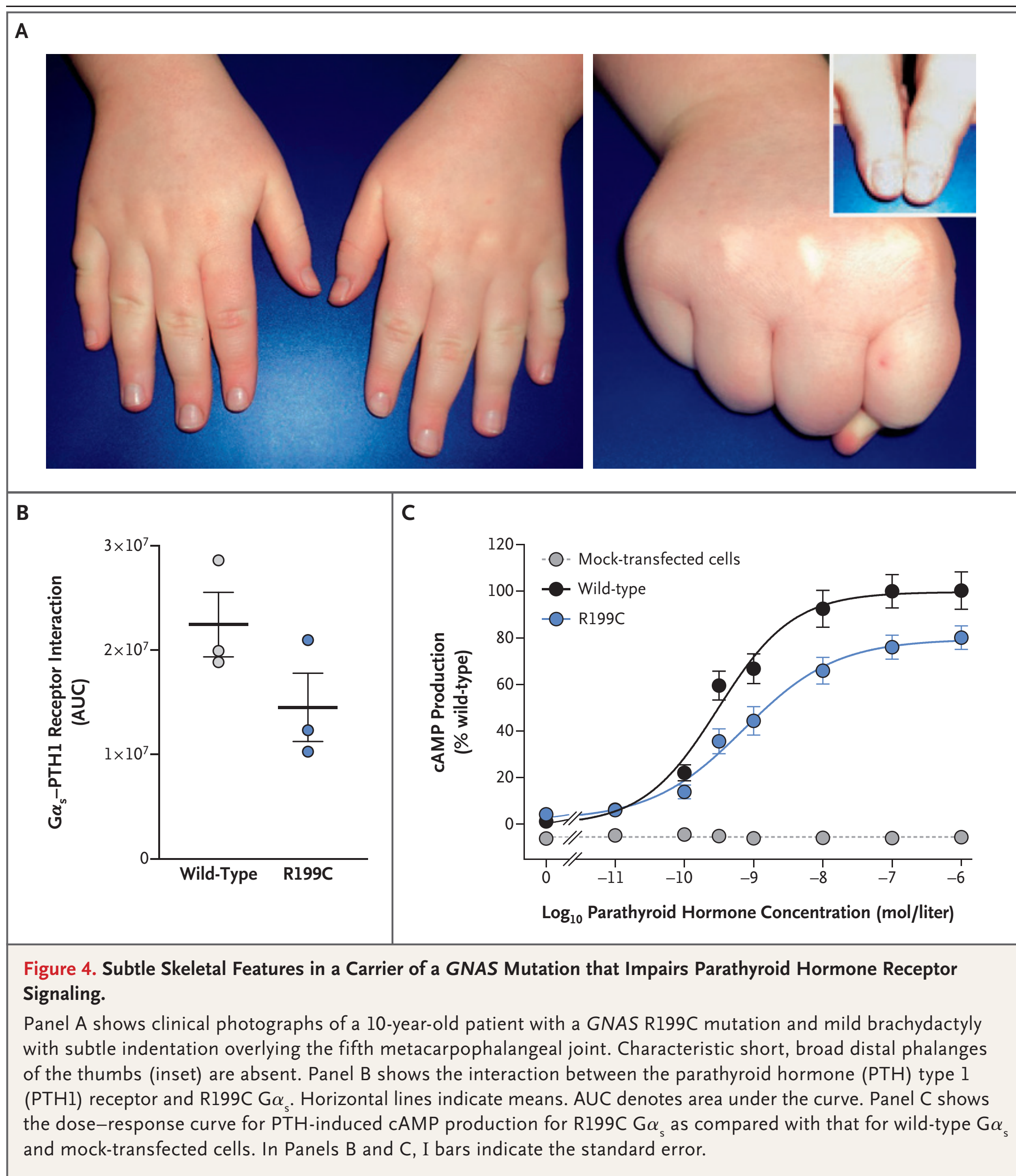

Figure 4. Subtle Skeletal Features in a Carrier of a *GNAS* Mutation that Impairs Parathyroid Hormone Receptor Signaling.

Panel A shows clinical photographs of a 10-year-old patient with a *GNAS* R199C mutation and mild brachydactyly with subtle indentation overlying the fifth metacarpophalangeal joint. Characteristic short, broad distal phalanges of the thumbs (inset) are absent. Panel B shows the interaction between the parathyroid hormone (PTH) type 1 (PTH1) receptor and R199C $G\alpha_s$. Horizontal lines indicate means. AUC denotes area under the curve. Panel C shows the dose–response curve for PTH-induced cAMP production for R199C $G\alpha_s$ as compared with that for wild-type $G\alpha_s$ and mock-transfected cells. In Panels B and C, I bars indicate the standard error.

pseudohypoparathyroidism appear, if they do at all.

Setmelanotide, an MC4R agonist, was recently approved for long-term weight management in patients with obesity syndromes that disrupt the melanocortin pathway[33-35]; our findings indicate that obesity in *GNAS* deficiency may similarly be treatable by MC4R agonists. Since *GNAS* mutations impaired signaling by β_2- and β_3-adrenoreceptors and by thyrotropin receptors, reduced energy expenditure, lipolysis, or both may also contribute to weight gain. *GNAS* mutations also impair both the direct interaction of $G\alpha_s$ with adenylyl cyclase 2, an isoform of adenylyl cyclase and a GPCR effector that is highly expressed in the hypothalamus (Fig. S8), and the signaling by hypothalamic $G\alpha_s$-coupled GPCRs.

Our clinical review revealed that although features of pseudohypoparathyroidism developed in some patients over time (Table 1 and Table S6), the diagnosis was not considered in others. Thyrotropin resistance was an early feature in

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our series of patients, whereas hypocalcemia and PTH resistance emerged over time, which is consistent with previous series.[36,37] In our study, only 1 of the 10 patients who reached final height and who had *GNAS* deficiency had short stature, as compared with 60% of adults with pseudohypoparathyroidism type 1A.[38] We suggest a number of explanations for these observations. In patients presenting with pseudohypoparathyroidism, the mutational spectrum predominantly includes frameshift, nonsense, or splice-site mutations, with missense mutations in 30% of patients.[39] In contrast, in our study involving patients with severe obesity, 86% carried missense mutations. Furthermore, we found that the effect of *GNAS* mutations on GPCR signaling can explain considerable clinical variability. For example, six mutations impaired PTH receptor signaling without affecting GHRH receptor signaling, causing PTH resistance but not short stature. We speculate that these differences may be partly due to the fact that the GHRH receptor has a shorter C-terminal tail than does the PTH receptor (Fig. S9), and thus it may be less likely to interact with a mutant form of $G\alpha_s$.

As more patients undergo unbiased genetic testing (with exome sequencing and gene panels), revealing a broader spectrum of mutations in disease-associated genes, the range of clinical features associated with monogenic diseases is likely to expand. The discovery that variants initially described as having uncertain significance are actually pathogenic and the linkage of molecular findings with clinical data provide a tremendous opportunity to enhance understanding of disease mechanisms, accelerate diagnosis, and inform the clinical care of a substantial number of patients with single-gene disorders.

Supported by grants from Wellcome (207462/Z/17/Z), the National Institute for Health Research (NIHR) Cambridge Biomedical Research Centre, Fondation Botnar, and the Bernard Wolfe Health Neuroscience Endowment and by an NIHR Senior Investigator Award (all to Dr. Farooqi); an award from the Expanding Excellence in England Fund from Research England (to Dr. Barroso); and a Wellcome Trust Major Award (208363/Z/17/Z, to the Wellcome–Medical Research Council Institute of Metabolic Science Translational Research Facility).

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

We thank the physicians who referred patients to the Genetics of Obesity Study and the patients and families for their involvement.

APPENDIX

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