BRIEF REPORT

Rare Antagonistic Leptin Variants and Severe, Early-Onset Obesity

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SUMMARY

Hormone absence or inactivity is common in congenital disease, but hormone antagonism remains controversial. Here, we characterize two novel homozygous leptin variants that yielded antagonistic proteins in two unrelated children with intense hyperphagia, severe obesity, and high circulating levels of leptin. Both variants bind to the leptin receptor but trigger marginal, if any, signaling. In the presence of nonvariant leptin, the variants act as competitive antagonists. Thus, treatment with recombinant leptin was initiated at high doses, which were gradually lowered. Both patients eventually attained near-normal weight. Antidrug antibodies developed in the patients, although they had no apparent effect on efficacy. No severe adverse events were observed. (Funded by the German Research Foundation and others.)

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Leptin serves as a signal of energy sufficiency in the brain, where a critically low level of the hormone triggers behavioral, metabolic, and endocrine responses that aim at restoring and preserving energy reserves.[1] Leptin acts by binding to the long isoform of the leptin receptor (LEPRb),[2] eliciting various signaling events, including phosphorylation of signal transducer and activator of transcription 3 (STAT3).[2]

Congenital leptin deficiency and dysfunction are rare, autosomal recessive forms of severe, early-onset obesity caused by changes in the leptin gene (*LEP*; gene identification number, 3952).[3-6] To date, 21 distinct variants have been described (Table S1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).[7] Most of the variants cause defects in production or secretion and result in complete hormone deficiency,[3,4,7] although a few variants result in impaired receptor binding and hormone dysfunction.[5,6] Intense hyperphagia and impaired satiety develop in affected persons, leading to rapid weight gain and severe obesity with hyperinsulinemia, hyperglycemia, dyslipidemia, and hepatic steatosis.[7] These persons generally have hypogonadotropic hypogonadism, delayed pubertal development, and recurrent severe infections.[7] The disease can be treated efficiently with recombinant leptin.[8]

METHODS

CASE REPORTS

We describe two unrelated children, a 14-year-old boy (Patient A) and a 2-year-old girl (Patient B), who had characteristics of leptin dysfunction including intense hyperphagia, impaired satiety, and severe, early-onset obesity (see the Supplementary Appendix).[7] The parents of Patient A are second-degree cousins of European descent; the parents of Patient B are first-degree cousins of Arab descent. Written informed consent was obtained from the parents of each child, and procedures were approved by the local ethics committees. The identified leptin variants were submitted to the ClinVar repository under accession numbers SCV003761543 and SCV003761542. The funding bodies had no role in the design or conduct of the study; the collection, management, analysis, or interpretation of the data; the preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication.

LEPTIN PRODUCTION AND SECRETION STUDIES

Nonvariant and variant leptins were produced in human embryonic kidney 293 (HEK293) cells by transient transfection. Culture medium and cell lysates were analyzed by means of Western blotting with a polyclonal antileptin antibody. Nonvariant and variant leptin–mCherry and leptin–NLuc fusions were generated to study leptin-receptor binding and internalization.

LEPTIN RECEPTOR OVEREXPRESSION AND FUNCTIONAL STUDIES

HEK293, COS7, and MCF7 cells overexpressing LEPRb were generated by transient transfection or stable viral transduction. Details on protein structure modeling, activity, binding, internalization, and competition studies are provided in the Supplementary Appendix.

STATISTICAL ANALYSIS

Statistical analyses were performed with the use of Prism software, version 8.4.2 (GraphPad). All P values are two-sided.

RESULTS

DISCOVERY OF NOVEL LEPTIN VARIANTS

Both patients had high levels of circulating leptin, a finding consistent with their high fat mass (Table 1 and Table S2). After Prader–Willi and Bardet–Biedl syndromes were ruled out, *LEP* was sequenced. Patient A had a homozygous cytosine-to-thymine transition at complementary DNA (cDNA) position 190 (NM_000230.3:c.190C→T), which is predictive of a proline-to-serine exchange (NP_000221.1:p.Pro64Ser [P64S]). Patient B had a homozygous guanine-to-adenine transition at cDNA position 175 (NM_000230.3:c.175G→A), which is predictive of a glycine-to-serine exchange (NP_000221.1:p.Gly59Ser [G59S]). Both variants can be classified as pathogenic (see the Supplementary Appendix).[10] The parents of each patient were heterozygous for the variants.

CHARACTERIZATION OF LEPTIN VARIANTS

We performed in vitro experiments to investigate whether the identified leptin variants were involved in the development of the disease. After transient overexpression in HEK293 cells, leptin variants P64S and G59S were detected in cell lysates and supernatants, each in an amount similar to that of nonvariant leptin (Fig. 1A). LEPRb-overexpressing HEK293 (HEK293–LEPRb) cells were then exposed to supernatants of HEK293 cells transfected with nonvariant and variant leptin. Whereas treatment with nonvariant leptin robustly increased STAT3 phosphorylation and transcriptional activity, treatment with leptin variants P64S and G59S resulted in a marginal response (Fig. 1B and 1C).

We then probed the interaction between the P64S and G59S variants and the leptin receptor. In the absence of leptin, LEPRb might exist as preformed oligomers on the cell surface.[11-14] Leptin features three interaction sites (IS-I, IS-II, and IS-III) through which it engages the leptin receptor, forming an active oligomeric complex.[15] IS-II and IS-III are critical for the bioactivity of leptin.[16-19] IS-II is formed by residues of helixes A and C and mediates high-affinity binding to the second cytokine-receptor homology domain (CRH2) of one LEPRb chain to establish an initial leptin–LEPRb complex.[20-22] IS-III comprises the AB loop and parts of helix D and subsequently partakes in a low-affinity interaction with the immunoglobulin-like domain (IGD) of another LEPRb chain to induce conformational changes in the complex, causing its activation.[15] Afterward, additional LEPRb chains may also be recruited to the complex.[15] Thus, synergistic IS-II–CRH2 and IS-III–IGD interactions are

Table 1. Anthropometric Measures and Serum Concentrations of Selected Endocrinologic and Metabolic Markers in the Patients before and after Initiation of Treatment with Metreleptin.*

Variable	Reference Range	Timeline of Therapy							
		Day −6	Day 8	Day 15	Day 40	Day 131	Day 264	Day 355	Day 1188
Patient A, carrier of leptin variant P64S									
Age (yr)	—	14.65	14.69	14.71	14.78	15.03	15.39	15.64	17.92
BMI	—	54.3	53.3	52.4	48.3	38.0	31.2	29.8	26.9
BMI-SDS	−2.0 to 1.0	5.87	5.73	5.59	5.02	3.55	2.62	2.38	1.51
Metreleptin dose (mg/kg of LBW)	—	—	0.70	0.70	0.70	0.31	0.15	0.15	0.15
Leptin (ng/ml)	—	52	NA	153	107	78	92	19	3
Hunger score†	—	5.58	NA	2.93	2.90	NA	1.80	2.50	3.53
Fasting glucose (mg/dl)	46.8 to 99.1	95.5	95.5	82.9	77.5	68.5	82.9	82.9	93.7
Fasting insulin (mU/liter)	2 to 31	52	37	32	24	6	12	12	6
Enzymes (U/liter)									
Alanine aminotransferase	<30	64	81	120	62	21	20	24	19
Aspartate aminotransferase	<30	39	53	66	48	24	21	24	19
γ-Glutamyltransferase	10 to 22	31	27	28	24	22	29	40	30
		Day −1	Day 8	Day 20	Day 44	Day 135	Day 238	Day 324	Day 1260
Patient B, carrier of leptin variant G59S									
Age (yr)	—	2.26	2.29	2.32	2.39	2.64	2.92	3.16	5.72
BMI	—	31.6	NA	28.8	25.1	20.2	17.4	19.1	17.6
BMI-SDS	−2.0 to 1.0	8.92	NA	7.46	5.48	2.91	1.39	2.39	1.31
Metreleptin dose (mg/kg of LBW)	—	—	0.15	0.15	0.15	0.15	0.09	0.08	0.07
Leptin (ng/ml)	—	27	NA	14	136	NA	218	22‡	16‡
Hunger score	—	NA	NA	NA	NA	NA	NA	NA	NA
Fasting glucose (mg/dl)	46.8 to 99.1	88.3	104.5	93.7	82.9	NA	NA	NA	88.3
Fasting insulin (pmol/liter)	2 to 31	6	7	6	3	NA	5	4	6
Enzymes (U/liter)									
Alanine aminotransferase	<30	38	NA	33	33	29	31	18	11
Aspartate aminotransferase	<30	26	NA	23	16	15	19	36	31
γ-Glutamyltransferase	10 to 22	10	NA	9	8	7	7	9	9

* To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for insulin to picomoles per liter, multiply by 7.175. BMI-SDS denotes body-mass index (the weight in kilograms divided by the square of the height in meters) standard deviation score, LBW lean body weight, and NA not assessed.

† The hunger score was determined with the use of a questionnaire developed by Hill and Blundell,[9] which is composed of six questions (e.g. "How strong is your desire to eat?") and presented on a visual-analogue scale ranging from 0 to 10 (e.g., from "I have no desire to eat" to "I have a very strong desire to eat"). . For questions 1 through 5, higher values indicate increased hunger. For question 6, a higher value indicates decreased hunger. The final hunger score represents the mean of the values obtained for questions 1 through 5 and for question 6 (calculated as 10 minus the value). The hunger score was assessed in Patient A only, who was old enough to use a visual-analogue scale. The questionnaire was presented to the patient in the morning after an overnight fast.

‡ The last two circulating leptin measurements for Patient B were performed with an enzyme-linked immunosorbent assay kit from a different supplier.

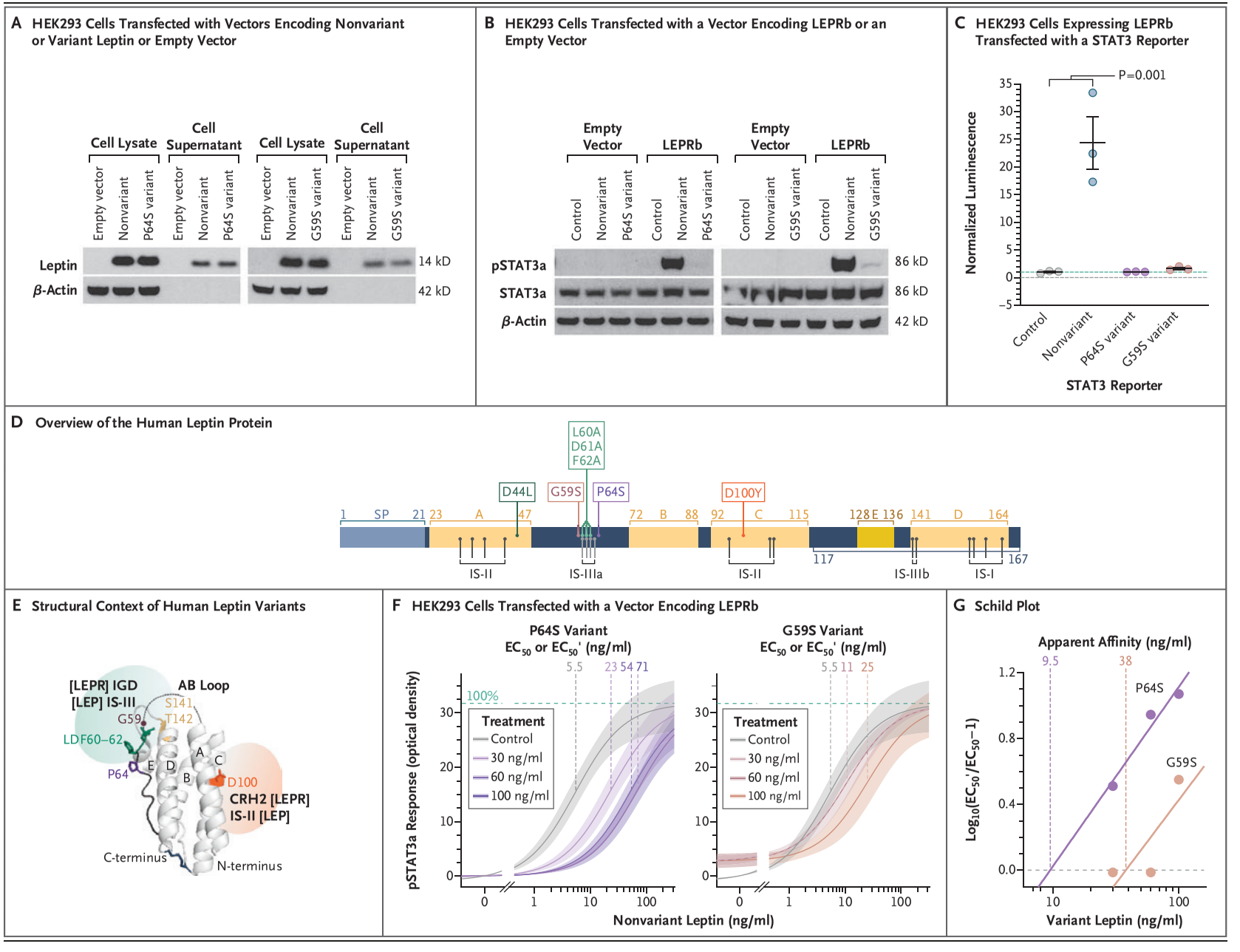


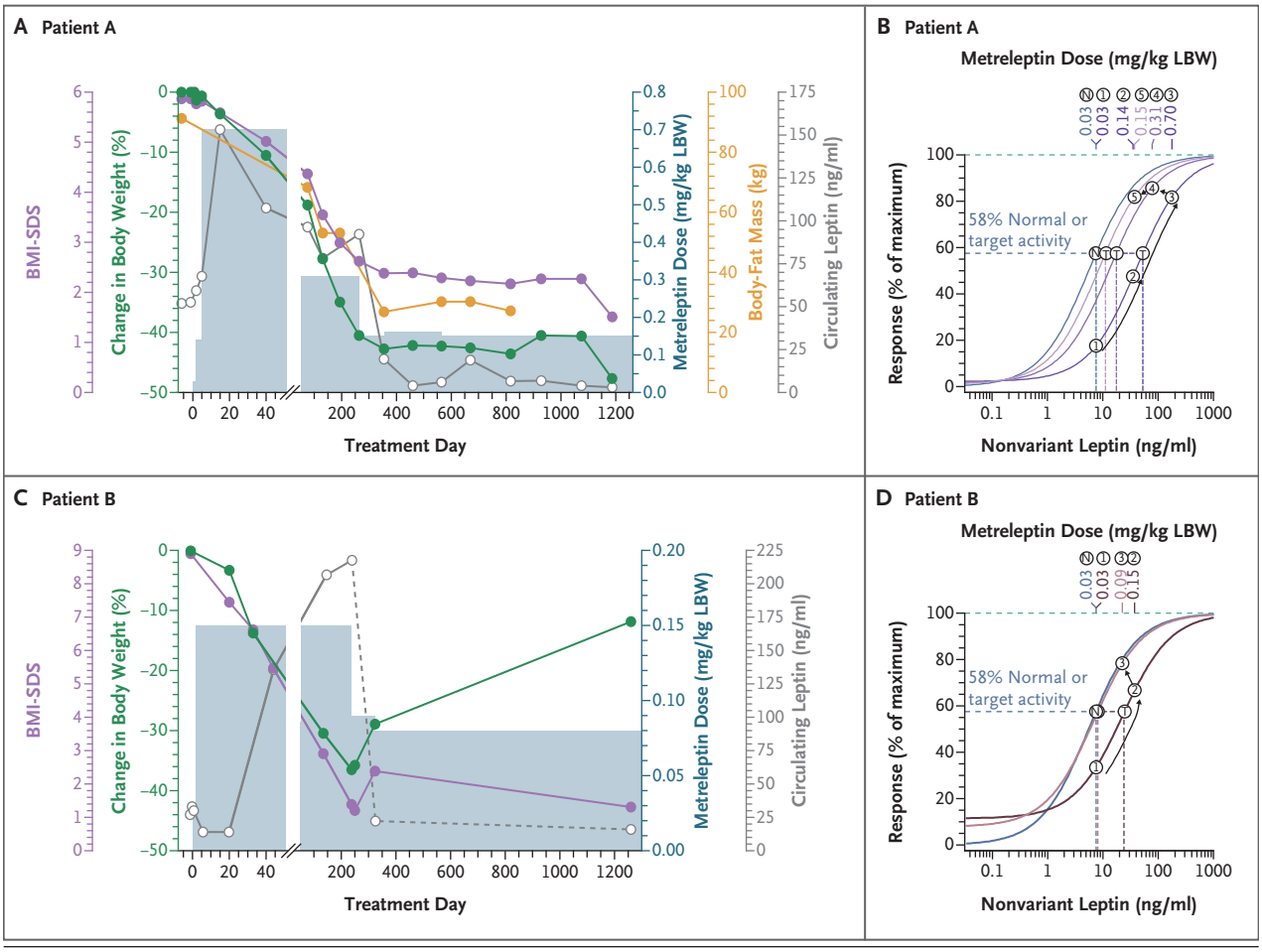
Figure 1 (facing page). Characterization of Leptin P64S and G59S.
Panel A shows transfection of human embryonic kidney 293 (HEK293) cells with vectors encoding nonvariant leptin, variant leptins (P64S and G59S), or a corresponding empty vector. Leptin was detected in cell lysates and supernatants by Western blot analysis. β-Actin served as a loading control. Three independent experiments were performed. Panel B shows transfection of HEK293 cells with a vector encoding the long isoform of the human leptin receptor (LEPRb) or a corresponding empty vector and treatment with supernatants containing 30 ng per milliliter of nonvariant leptin or variant leptins (P64S and G59S). Supernatant from empty vector–transfected cells was used as a control. Phosphorylated signal transducer and activator of transcription 3a (pSTAT3a, Y705) and STAT3a were analyzed by means of Western blotting. β-Actin served as a loading control. Three independent experiments were performed. Panel C shows transfection of HEK293 cells stably expressing LEPRb with a STAT3 reporter and treatment with supernatants containing 100 ng per milliliter of nonvariant leptin or variant leptins (P64S and G59S). HEK293 cell-conditioned supernatant was used as a control. Data were normalized to the control condition and are displayed as the mean (horizontal line) with standard error (I bar) of three independent experiments. A one-way analysis of variance with Dunnett-corrected multiple comparisons was performed. Panel D shows an overview of the human leptin protein. Numbers indicate amino acid positions. Shown are the locations of the N-terminal signal peptide (SP) that gets cleaved off during protein synthesis, the four major α helixes A to D, the minor distorted α helix E, the single disulfide bond, and the three interaction sites (IS-I, IS-II, and IS-III) that have been implicated in leptin receptor engagement. Similarly, the positions of naturally occurring (P64S, G59S, and D100Y) and rationally designed (D44L and LDF60-62AAA) variants are marked. Panel E shows the structural context of human leptin variants. Leptin (Protein Data Bank identifier 1AX8) is shown with the aforementioned α helixes A through E. The AB loop (residues 48 to 71) connecting α helixes A and B is shown in black. AB loop residues 48 to 59 were unmodeled in the determined crystal structure and are represented by a thin dashed line. Leptin (LEP) residues contributing to IS-II map to α helixes A and C and mediate a high-affinity interaction with the second cytokine receptor homology domain (CRH2) of the leptin receptor (LEPR), while LEP residues located in IS-III (G59, L60, D61, F62, P64, and S141/T142) contribute to a low-affinity interaction with the immunoglobulin-like domain (IGD) of another LEPR chain to drive complex activation. Panels F and G show the treatment of LEPRb-transfected HEK293 cells with supernatants containing 0 to 100 ng per milliliter of nonvariant leptin as well as 30, 60, or 100 ng per milliliter of variant (P64S or G59S) leptin. Supernatants from empty vector–transfected cells were used as a control. Western blotting was used in the analysis of pSTAT3a (Y705) and STAT3a, and densitometric analysis was performed with the use of ImageJ software (National Institutes of Health). Data are displayed as means with 95% confidence intervals (in shaded areas) of four independent experiments for each variant leptin. As shown in Panel F, the data were subjected to global nonlinear least-squares curve fitting to a three-parameter Hill equation. Details are provided in the Supplementary Appendix. The half maximal effective concentration values (dashed colored vertical lines) of nonvariant leptin that were determined in the absence (EC_{50}) or presence (EC_{50}') of the variants are indicated. In the graphs, 5.5 ng per milliliter is the EC_{50} value, and 23, 54, and 71 ng per milliliter (left graph) and 11 and 25 ng per milliliter (right graph) are the EC_{50}' values. Panel G shows a Schild plot generated by plotting the concentrations of the leptin variants against a parameter generated from the EC_{50} and EC_{50}' values. The apparent affinities (dashed colored vertical lines) of the variants to LEPR are indicated.

required for LEPRb signaling. Mutagenesis revealed that changes in IS-II residues greatly reduce LEPRb binding, whereas changes in IS-III residues reduce LEPRb signaling.[16-19]

P64 and G59 are part of the AB loop that connects the α helixes A and B of leptin (Fig. 1D and 1E). They flank the IS-III residues p.Leu60_Phe62, the mutagenesis of which yielded the antagonistic variants p.Leu60_Phe62delinsAlaAlaAla (AAA)[16] and p.Asp44Leu;Leu60_Phe62AlaAlaAla (L+AAA).[23] The AB loop adopts a flexible structure,[24] and it was proposed that the AB loop gains order only on receptor binding.[20-22] Within functional loop segments, proline and glycine residues bestow critical conformational properties. The change of these residues to serine in leptin variants P64S and G59S may thus affect the ability of the AB loop to properly position IS-III residues for the IS-III–IGD interaction, thereby affecting the activation but not the formation of the leptin–LEPRb complex (Fig. 1E). In the presence of nonvariant leptin, leptin variants P64S and G59S could therefore act as competitive LEPRb antagonists.

We first studied the ability of the leptin variants to bind to LEPRb. To this end, we generated leptin–mCherry fusions that showed unaltered protein production, secretion, and activity (Fig. S1A and S1B). As was the case with nonvariant leptin and the antagonistic AAA[16] and L+AAA[23] variants, both leptin variants P64S and G59S bound to and were internalized with their receptor in HEK293–LEPRb cells (Fig. S1C to S1E). As expected,[5] we detected no binding in the case of the p.Asp100Tyr (D100Y) variant. Moreover, through an approach using leptin–NanoLuc fusions and COS7–LEPRb cells, the binding affinities for nonvariant and P64S and G59S variant leptins were measured and found to be identical (Fig. S2A). Biolayer interferometry measurements showed highly similar binding kinetics and affinities to the isolated leptin-receptor CRH2 fragment (Fig. S2B).

We next studied the ability of the leptin variants to interfere with LEPRb activation. HEK293–LEPRb cells were treated with increasing concentrations of nonvariant leptin in the presence of the variants (Fig. 1F and Fig. S3A). Both leptin variants P64S and G59S effectively suppressed nonvariant-induced STAT3 phosphorylation, shifting the concentration–response curve to the right, indicating competitive antagonism. In the assessment of the STAT3 response with a cell-based enzyme-linked immunosorbent assay, the



antagonistic capacity of the P64S variant was confirmed but was determined to be lower than that of leptin AAA and L+AAA (Fig. S3B). Similar patterns were observed for STAT1 phosphorylation in HEK293–LEPRb cells (Fig. S3C and S3D) and for STAT1 and STAT3 phosphorylation in MCF7–LEPRb cells (Fig. S4). As observed in these experiments, the antagonistic behavior was more pronounced for the leptin variant P64S than for G59S. Both variants also showed partial agonist activity in the absence of nonvariant leptin, which was subtle for the P64S variant but definite for the G59S variant (approximately 3% and 18%, respectively, relative to nonvariant leptin). It is important to note that in the presence of a full agonist such as nonvariant leptin, partial agonists behave as competitive antagonists.[25]

By calculating the relative affinities[26] of the leptin variants to their receptor on the basis of the STAT3 response, we found that the apparent affinities of the P64S and G59S variants were 1.7 times and 6.9 times, respectively, lower than that of nonvariant leptin (Fig. 1G). These lower apparent affinities might be caused by the partial agonist activity of the variants and the use of a signaling-based method that did not discriminate between decreases in binding affinity and increases in residual activity. In support of this notion, leptin G59S, which possesses a higher residual activity than leptin P64S, also shows a lower apparent affinity than leptin P64S. Complexities of receptor binding and activation mechanisms may have also contributed to the observed differences.[15,27] Collectively, these results show that leptin variants P64S and G59S behave as competitive LEPRb antagonists in the presence of nonvariant leptin, while retaining faint partial agonist activity in its absence.

TREATMENT OF PATIENTS

We initiated metreleptin treatment at a dose of 0.03 mg per kilogram of lean body weight, the recommended dose that is known to evoke immediate effects on food intake and satiety in pa-

Figure 2 (facing page). Hormone-Replacement Therapy in Patients A and B.

The patients were treated with recombinant human leptin (metreleptin). Panel A shows the change in body weight, the body-mass index (the weight in kilograms divided by the square of the height in meters) standard deviation score (BMI-SDS), the body fat mass, and the level of circulating leptin in Patient A (carrier of leptin variant P64S) during hormone-replacement therapy. LBW denotes lean body weight. Panel B shows the dose escalation and tapering of metreleptin in Patient A. The concentration–response curve obtained for nonvariant leptin is shown in blue. The concentration–response curve in the presence of a pretherapeutic P64S variant level of 52 ng per milliliter is shown in dark purple. A plasma concentration of 7.5 ng per milliliter, which is found in normal persons, was predicted to cause an activation of LEPR signaling by 58% of the maximum. It is presumed that this is the normal degree of activation (N) to be reached by hormone replacement, that is, the therapeutic target (T). Because activation of LEPR signaling is attained in patients with leptin deficiency[5,6,28] at a daily dose of 0.03 mg per kilogram of LBW (1), it was assumed that this dose would cause such a normal degree of receptor activation (N), which is taken as the therapeutic target (T) herein. Drug therapy was initiated at this dose, which was predicted to cause only 18% of maximal LEPR activation in the presence of the endogenous leptin variant. The dose was then increased to 0.14 mg per kilogram of LBW (2), followed by a further increase to 0.70 mg per kilogram of LBW (3), causing 48% and 82% of maximal LEPR activation, respectively. After 131 days, the patient had lost 49.9 kg of body weight, corresponding to a weight loss of 27.8%, and after 264 days, the patient had lost 72.9 kg of body weight, corresponding to a weight loss of 40.5%, which have previously been found to cause reductions of endogenous leptin levels[29] to approximately 30% and 10%, respectively. The corresponding concentration–response curves are shown in purple and light purple. The degrees of maximum LEPR activation reached with dose tapering to (4) and (5) were predicted to amount to 86% and 82%, respectively, of maximal LEPR activation. Panel C shows the change in body weight, the BMI-SDS, and the level of circulating leptin in Patient B (carrier of leptin G59S) during hormone-replacement therapy. The last two circulating leptin measurements were performed with an enzyme-linked immunosorbent assay kit from a different supplier. Panel D shows the dose escalation and tapering of metreleptin in Patient B. The concentration–response curve in the presence of a pretherapeutic G59S variant level of 27 ng per milliliter is shown in dark red. The extent of LEPR activation at the beginning of hormone replacement with 0.03 mg per kilogram of LBW (1) was predicted to cause 32% of maximum activation. In this case, a single increase of the dose to 0.15 mg per kilogram of LBW (2) was expected to cause a rise in receptor activation to 66%, which was sufficient to cause therapeutic effects. After 238 days, the patient had lost 9.6 kg of body weight, corresponding to a weight loss of 36.5%. Such weight loss has previously been found to cause a reduction of the endogenous leptin level[29] to approximately 25%. The corresponding concentration–response curve is shown in light red. The degree of maximum LEPR activation reached on tapering of the dose to (3) was predicted to amount to 86% of maximal LEPR activation.

tients with congenital leptin deficiency or dysfunction (Fig. 2A and 2C).[5,6,8] This initial treatment had no apparent effect on food intake or satiety (Table 1), a finding indicating that higher-than-usual doses would be necessary to overcome the antagonism of the endogenous variants.

Guided by our in vitro characterization, we performed dose escalation and tapering (Fig. 2B and 2D; see also the Supplementary Appendix). For Patient A, the dose was increased to 0.14 mg per kilogram on day 2 of treatment and to 0.70 mg per kilogram on day 5. In Patient B, the dose was increased to 0.15 mg per kilogram on day 2 of treatment. In addition, both patients participated in vigorous fasting and exercise programs to lower endogenous leptin production and enhance the therapeutic effects of exogenous leptin. This approach resulted in steep rises in circulating leptin levels, which reflect combined endogenous and exogenous leptin, and eventually evoked a therapeutic response, with a normalization of food intake and satiety and weight loss. As anticipated,[30] antibodies against metreleptin developed in both patients, which may have added to the observed increase in circulating leptin levels. Antileptin antibodies are thought to delay the clearance and thus increase the half-life of leptin in the circulation.[30] However, no loss of metreleptin efficacy was observed in either patient at any time, a finding that argues against the occurrence of clinical leptin resistance.

Over the course of treatment, Patient A had a marked reduction in fat mass and attained a near-normal weight of 94.1 kg (body-mass index [the weight in kilograms divided by the square of the height in meters] standard deviation score [BMI-SDS], 1.51) after 1188 days of treatment. We observed a transient rise in aminotransferase and insulin levels (Fig. S5A and S5B and Table 1), findings similar to observations in patients with leptin deficiency treated with much lower doses of metreleptin.[6,31] Patient A showed pubertal progression to a mature Tanner stage (pubic hair, PH5; testicular and penile development, G5; and testicular volume, 15 to 20 ml) at 16.5 years of age. Patient B similarly attained a near-normal weight of 23.2 kg (BMI-SDS, 1.31) after 1260 days of treatment.

After the two patients had extensive weight loss, which was presumably accompanied by reductions in the endogenous production of leptin variants, we reduced the metreleptin dose in both patients. Taken together, the newly identi-

fied P64S and G59S variants caused a novel form of congenital leptin dysfunction characterized by hormone antagonism, which created a distinct challenge in the early phase of treatment.

DISCUSSION

Pathogenic changes in *LEP* commonly result in classical hormone deficiency.[3,4,7] We previously described rare, secreted — but biologically inactive — leptin variants that cause hormone dysfunction due to a lack of receptor binding.[5,6] This discovery changed the diagnostic algorithm applied to severe, early-onset obesity, from a sole assessment of the presence of leptin in the circulation of patients[32] to an additional determination of its receptor-binding capacity.[29]

The new antagonistic variants described herein add further complexity to the diagnosis and treatment of congenital leptin dysfunction. The phenotypes of patients with antagonistic and biologically inactive leptin variants are indistinguishable from one another before hormone replacement, although the phenotypes diverge after the administration of metreleptin. Whereas patients with biologically inactive variants have a good response to low metreleptin doses, similar to the response in those with classical leptin deficiency,[5,6] higher-than-usual doses are necessary in patients with antagonistic variants so that the competitive antagonism of the endogenous hormone may be overcome. This is a problem particular to the early phase of hormone-replacement therapy, when the circulating variant levels are still high. Once an appreciable therapeutic response can be evoked and the endogenous leptin production has been sufficiently reduced by a loss of fat mass and negative feedback of active leptin signaling on fat cell leptin secretion, the late phase of therapy can proceed at much lower doses of metreleptin.

Inactive hormone variants were previously described for growth hormone,[33-35] thyrotropin,[36] luteinizing hormone,[37] insulin,[38] and insulin-like growth factor I.[39] Hormone antagonism, in contrast, has thus far been claimed only for growth hormone[33,34] and has remained controversial[40,41] or formally unproven (e.g., until interference with nonvariant-induced signaling is shown).[34] Here, we provide in vitro and in vivo evidence for the existence of leptin variants that orthosterically bind to but do not fully activate the leptin receptor and hence act as competitive antagonists in the presence of nonvariant leptin. Recently obtained structural insights into the assembly of leptin–LEPRb complexes through IS-III[15] rationalize the antagonistic behavior of the P64S and G59S leptin variants.

Our work exemplifies how thorough in vitro characterizations can make decisive contributions to overall therapeutic success. Functional characterization of the protein, in addition to genotyping, helps in the discrimination of leptin variants that display defects in synthesis, secretion, receptor binding, or receptor activation. The correct diagnosis of classical leptin deficiency or leptin dysfunction with inactive or antagonistic variants is a requisite for the personalized treatment of affected patients.

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APPENDIX

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