BRIEF REPORT

# Variant Prolactin Receptor in Agalactia and Hyperprolactinemia

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## SUMMARY

A loss-of-function variant in the gene encoding the prolactin receptor (*PRLR*) was reported previously in a woman with persistent postpartum galactorrhea; however, this paradoxical phenotype is not completely understood. Here we describe a 35-year-old woman who presented with idiopathic hyperprolactinemia that was associated with a complete lack of lactation after each of her two deliveries. She is a compound heterozygote for loss-of-function variants of *PRLR*. Her unaffected parents are heterozygotes. These findings are consistent with previous work showing that mice deficient in functional *Prlr* do not lactate.

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Prolactin, a pituitary hormone, plays diverse roles in vertebrates; its known roles in humans are in the processes of lactation and reproduction.[1,2] A loss-of-function variant in the gene encoding the prolactin receptor (*PRLR*) has been reported in a proband with persistent postpartum galactorrhea and hyperprolactinemia.[3,4] The proband and her two sisters were heterozygous for a missense variant (H212R). The proband received dopamine agonist therapy to terminate the persistent galactorrhea after all (four) of her deliveries. She and a sister were oligomenorrheic; her other sister was infertile.

The study[3] involving these sisters raises questions. Did the loss-of-function H212R variant cause postpartum galactorrhea?[2,4-7] Are infertility and oligomenorrhea the consequences of *PRLR* mutation or coincidental symptoms?[6] The phenotype of the proband was not observed in mice that were heterozygous for a knockout allele: $Prlr^{-/+}$ mice are normoprolactinemic, whereas $Prlr^{-/-}$ mice are hyperprolactinemic.[2,8] Does the allelic regulation of prolactin differ between mice and humans? To shed light on these questions, we report data regarding a female proband with biallelic loss-of-function *PRLR* variants and agalactia that was associated with hyperprolactinemia. We also report data on the proband's parents and two siblings (a sister and a brother).

## CASE REPORT

The proband was a 35-year-old woman (gravida 2, para 2), with normal menses (Fig. 1A). At 28 years of age, she sought the help of a gynecologist after 1 year of being unable to conceive and received a diagnosis of hyperprolactinemia (prolactin level, 128 ng per milliliter). She did not have galactorrhea. Pregnancy was successfully achieved on second intrauterine insemination. Ultrasonographic examination at 28 weeks of gestation revealed a fetus large for the gestational age (2.9 SD above the mean) and mild polyhydramnios. Normal results were obtained on a 75-g oral

glucose tolerance test (see the Supplementary Appendix, available with the full text of this article at NEJM.org). The polyhydramnios spontaneously resolved before 36 weeks of gestation.

The proband delivered a healthy boy (weight, 3766 g) by means of cesarean section at 37 weeks of gestation owing to placenta previa. Throughout the postpartum and puerperal periods, she had neither breast tension nor galactorrhea. Regular menstrual cycles that were associated with ovulation resumed 14 weeks after the delivery. The proband became pregnant at 17 months after the first delivery, without any infertility treatment. Her pregnancy was uneventful, and she delivered a healthy girl (weight, 3340 g) at 38 weeks of gestation. Again, she had no breast tension or galactorrhea. Ovulation-associated menstruation resumed 8 weeks after the second delivery. She was referred to our hospital for examination because her post-delivery prolactin levels were persistently high (188 to 255 ng per milliliter).

A thyrotropin-releasing hormone loading test revealed a high basal level of prolactin together with an excessive response (Fig. 1B); standard laboratory test findings (except the finding of lipidemia) were normal (Table S2 in the Supplementary Appendix). Standard screening for high-molecular-weight forms of prolactin (macroprolactin) with polyethylene glycol precipitation showed that macroprolactin accounted for 29% of the circulating prolactin, which is within the normal range of less than 40%. Magnetic resonance imaging revealed no evidence of a pituitary tumor (Fig. S2 in the Supplementary Appendix).

The proband's mother (61 years of age) was healthy at presentation and reported that she did not have and had not previously had menstrual irregularities or infertility. She had breast-fed each of her three children. She reported that she had been concerned about insufficient production of breast milk and had supplemented breast-feeding with synthetic milk. Lactation had ceased spontaneously within 3 months after each childbirth. The proband's father (69 years of age) was also fertile and healthy, except for mild hypertension. The serum prolactin levels of the proband's mother and father were 11 and 12 ng per milliliter, respectively. The proband's younger sister (25 years of age) was healthy and was pregnant (18 weeks of gestation), with a prolactin level of 73 ng per milliliter at presentation. The proband's younger brother (30 years of age) was also healthy, with a prolactin level of 9 ng per milliliter.

## METHODS

### STUDY OVERSIGHT

This study was approved by the institutional review board (Biomedical Research Ethics Committee at the Graduate School of Medicine, Chiba University). Written informed consent was obtained from each family member included in the study.

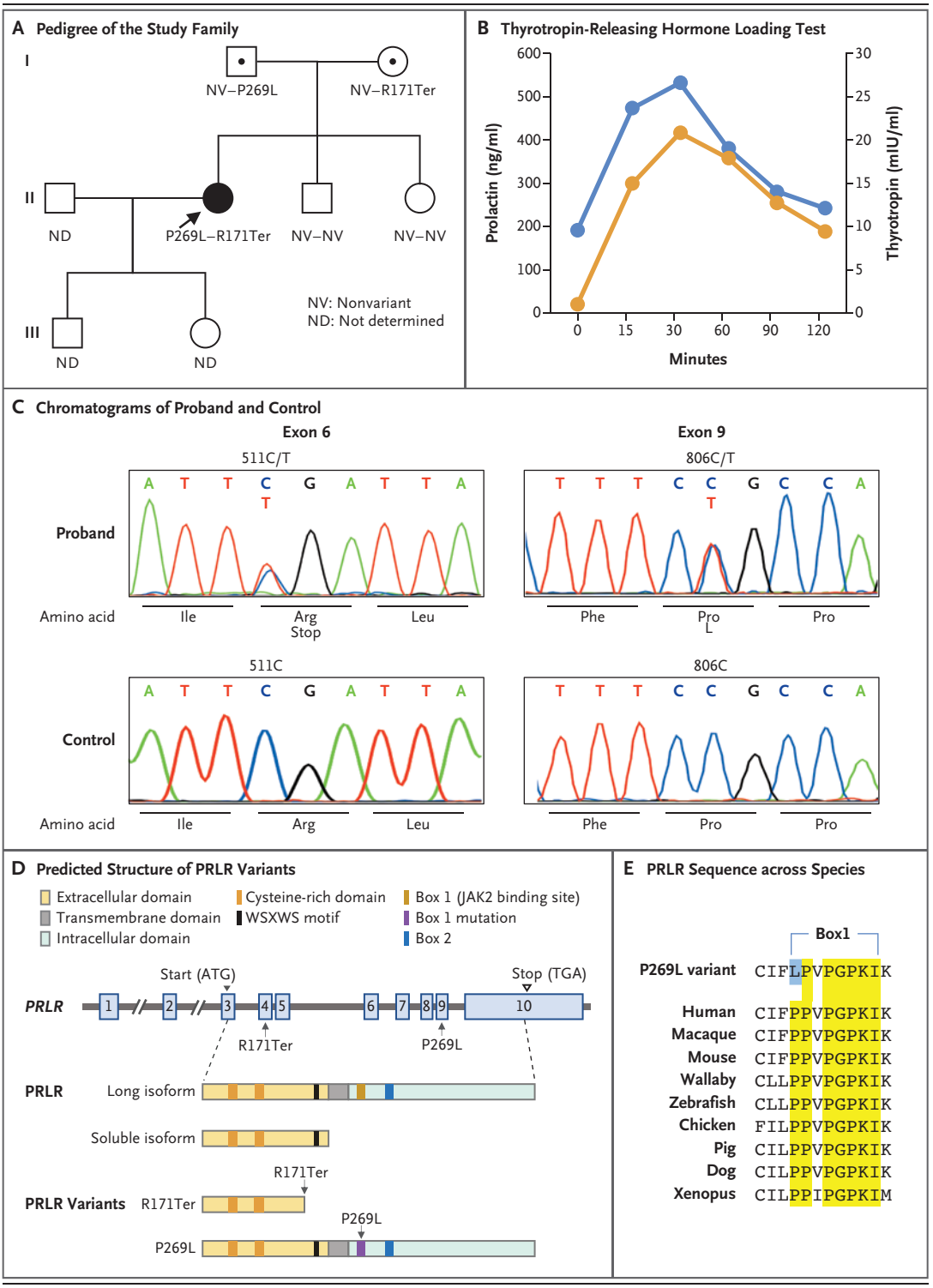
### LABORATORY METHODS

Genomic DNA was extracted from peripheral-blood samples, and the 11 exons of *PRLR* were amplified by means of a polymerase-chain-reaction assay and directly sequenced by means of Sanger sequencing (Table S1 in the Supplementary Appendix). We transfected human elongation factor 1$\alpha$ *PRLR–GFP* vectors carrying variant *PRLR* into human embryonic kidney (HEK) 293T cells. We localized the transfected prolactin receptor at the cellular level using antihuman PRLR antibody and a confocal fluorescence microscope. Phosphorylation responses to prolactin were assessed by means of Western blotting (see the Supplementary Appendix). STAT5-dependent gene expression was assayed with the use of a pGL4.10 luciferase reporter vector carrying the promoter region of the cytokine-inducible SRC homology 2 domain protein (CISH).[9]

## RESULTS

### GENOTYPING

The proband was a compound heterozygote for two cytosine-to-thymine transitions: one in exon 6 (NM_000949.6:c.511C→T; GenBank accession number, MH175194) and the other in exon 9 (NM_000949.6:c.806C→T; GenBank accession number, MH175193) of *PRLR*; these transitions predict nonsense (R171Ter) and missense (P269L) changes, respectively, at the amino-acid level (Fig. 1C and 1D). We are unaware of previous identification of R171Ter. P269L has been reported as a single-nucleotide variant (rs754974807) with a minor allele frequency of 0.00002[10]; however, the clinical significance of this variant is unclear.




The proband's mother and father were heterozygous for R171Ter and P269L, respectively. The proband's sister and brother had neither variant.

## FUNCTION OF MUTANT RECEPTORS

The 511C→T (R171Ter) variant gave rise to a premature termination codon, which resulted in a short polypeptide comprising the N-terminal of

**Figure 1 (facing page). Loss-of-Function Variants in the Gene Encoding Prolactin Receptor (*PRLR*) That Are Associated with Hyperprolactinemia.**

Panel A shows the pedigree of the family, with *PRLR* genotypes identified by bidirectional sequencing. Squares indicate male persons, and circles female persons. The arrow indicates the proband. The open symbols, dotted symbols, and solid symbols represent nonvariant, heterozygote, and compound heterozygote for mutations, respectively. Panel B shows serum levels of prolactin (blue) and thyrotropin (orange) after loading 500 μg of synthetic thyrotropin-releasing hormone. Panel C shows chromatograms obtained by direct sequencing of polymerase-chain-reaction products. Arg denotes arginine, Ile isoleucine, Leu leucine, Phe phenylalanine, and Pro proline. Panel D shows a schematic representation of the *PRLR*, full-length complementary DNA, and protein-domain structure. Variants and the predicted effect on protein are also shown. Panel E shows the alignment of the PRLR sequence across species. The conserved sequence is indicated by a yellow background; the P269L variant is highlighted in blue.

an extracellular domain (Fig. 1D). The predicted peptide lacks a transmembrane domain and the intracellular domain required for intracellular signal transduction, correct folding, and cellular trafficking, but it has two disulfide-linked cysteines, which are predicted to confer on the peptide the ability to bind ligand. The peptide is predicted to be similar to the soluble prolactin-binding isoform comprising only an extracellular domain, which modulates the function of the full-length prolactin receptor.[1,11] We transfected HEK 293T cells with a R171Ter *PRLR*–containing plasmid to examine the function of the variant receptor. We detected it in the cytoplasm at a significantly lower level than that of the nonvariant receptor (Fig. 2A, and Fig. S1 in the Supplementary Appendix); we observed no signal in the cell membrane. We observed protein of the expected size, albeit at a lower level than that of the nonvariant receptor, with the use of Western blotting (Fig. 2B). The R171Ter mutant did not induce STAT5 phosphorylation in response to prolactin (Fig. 2C).

The 806C→T variant predicts a full-length protein with an amino-acid substitution (P269L) at the docking site for the protein Janus kinase 2 (box 1 motif), which is in the phylogenetically conserved proline-rich domain (Fig. 1E).[12] We detected the variant protein in both the cytoplasm and cell membrane (Fig. 2A). Assay by Western blotting showed an elevated level of expression (Fig. 2B) and an inability to phosphorylate STAT5 (Fig. 2C) as compared with the nonvariant prolactin receptor.

We queried interactions between variant and nonvariant prolactin receptors by means of cotransfection experiments. Neither the R171Ter-variant protein nor the P269L-variant protein suppressed STAT5 phosphorylation by the nonvariant receptor when equimolar amounts of plasmids were transfected (Fig. 2D). As expected, the coexpression of R171Ter-variant and P269L-variant prolactin receptors did not induce STAT5 phosphorylation in response to prolactin.

The response to prolactin was quantified by means of the CISH promoter assay. The R171Ter-variant prolactin receptor did not respond to prolactin, whereas the P269L-variant prolactin receptor responded in a blunted fashion to 100 to 1000 ng per milliliter of prolactin (Fig. 2E). This small increase that was mediated by P269L *PRLR*–containing plasmid was suppressed by replacing half the amount of this plasmid with an equimolar amount of R171Ter *PRLR*–containing plasmid to create a condition mimicking that in a compound heterozygote (Fig. 2E). We also evaluated the possibility that the variants had a dominant-negative effect on the nonvariant receptor. The P269L variant attenuated the response of the nonvariant receptor by approximately 20%, whereas the R171Ter and H212R variants showed limited attenuation (Fig. 2F).

## DISCUSSION

Here we describe a woman with compound heterozygous germline inactivating variants of *PRLR* and hyperprolactinemia associated with postpartum agalactia. Biochemical data indicate that the variants negatively affect the prolactin receptor signaling pathway. Although we report the results of a single patient who may have other genetic variants (other than those in *PRLR*) that affect lactation, our data are consistent with the notion that signal transduction by means of the prolactin receptor is essential for lactation in humans.

Our data are also consistent with possibility that reduced lactation during breast-feeding is caused by heterozygous loss-of-function variants. The proband's mother, who was heterozygous

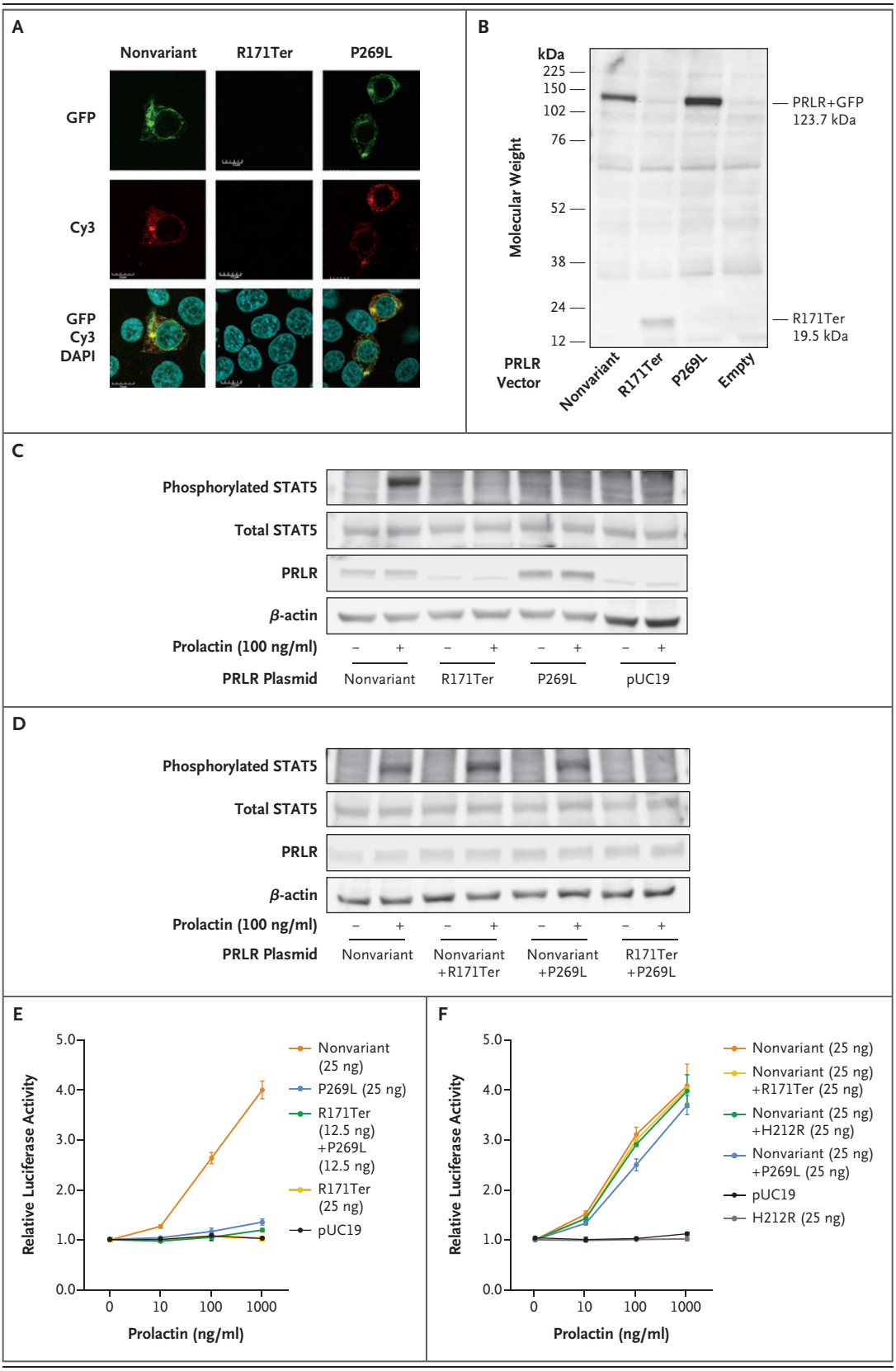


**Figure 2 (facing page). Expression and Function of the Variants in PRLR.**

Panel A shows expression of the prolactin receptor (PRLR). Human embryonic kidney (HEK) 293T cells were transiently transfected with a vector carrying nonvariant or variant *PRLR* sequences with a *GFP* tag (see the Supplementary Appendix). At 24 hours after transfection, the cells were fixed and incubated with an antibody against the human PRLR N-terminal (see the Supplementary Appendix) for another 24 hours and then with antirabbit antibody conjugated with Cy3 for 30 minutes. Confocal imaging detected the signals for green fluorescent protein (GFP) (top row) and Cy3 (middle row). The bottom row shows the superimposition of GFP, Cy3, and 4′,6-diamidino-2-phenylindole (DAPI) staining. Panel B shows a Western blot analysis of nonvariant and variant receptors expressed by HEK 293T cells. At 24 hours after transfection, HEK 293T cells were lysed and blotted with the use of anti-PRLR antibody (see the Supplementary Appendix). Panel C shows a Western blot analysis to detect STAT5 phosphorylation in HEK 293T cells on exposure to prolactin. HEK 293T cells were incubated with prolactin (100 ng per milliliter) for 30 minutes, 24 hours after transfection with plasmids carrying nonvariant *PRLR*, R171Ter *PRLR*, P269L *PRLR*, or pUC19 (1.6 μg DNA per well on a six-well plate). Panel D shows a Western blot analysis to detect functional interactions on the basis of STAT5 phosphorylation status after exposure to prolactin by HEK 293T cells expressing different types of PRLR (see the Supplementary Appendix). Panel E shows the dose–response curve of the gene encoding cytokine-inducible SRC homology 2 domain protein (CISH) promoter luciferase reporter in HEK 293T cells transfected with plasmids carrying nonvariant *PRLR*, variant *PRLR*, or pUC19. The cells were transfected with nonvariant or variant *PRLR* plasmids (DNA, 25 or 12.5 ng per well on a 24-well plate), CISH reporter (50 ng per well), Renilla reporter plasmid (5 ng per well), and pUC19 (varying amounts to make up total of amount of DNA of 755 ng per well). After 24 hours, the cells were stimulated with various amounts of prolactin for another 24 hours, lysed, and subjected to luciferase assay. Panel F shows the results of a CISH assay to query the effect of variant receptor on the function of nonvariant receptor. For comparison, H212R, a previously reported variant of *PRLR*, was included. In Panels E and F, I bars represent standard deviations.

for the R171Ter variant, reportedly had "insufficient" lactation. Similarly, mice that are heterozygous for a *Prlr* null allele have shown diminished lactation. That being said, we cannot rule out the possibility that insufficient lactation by the proband's mother was a coincidental symptom and unrelated to the allelic status of *PRLR*.

Our data support the idea that loss of prolactin receptor signaling does not disturb fertility in humans. The proband's parents, who are heterozygotes, were fertile. The proband, who is a compound heterozygote, had normal gonadotropin secretion, had a normal menstrual cycle, and was fertile, although the first pregnancy was achieved by means of intrauterine insemination after 1 year of trying to conceive without medical assistance. Thus, biallelic loss of function of *PRLR* would not seem to cause infertility in humans, although it is possible that there was some residual function of the P269L variant — that is, it is possible that the proband was not completely null for *PRLR* function and that residual function was key to her being able to conceive. Female mice that are homozygous for null mutations affecting prolactin receptor signaling have multiple reproductive defects, including infertility.[13,14]

Excessive production of prolactin inhibits the secretion of gonadotropin and leads to anovulation, probably by suppressing kisspeptin-1 release from the kisspeptin-1 neurons that express the prolactin receptor in the hypothalami of mice and humans.[15] The proband had loss of signaling by the prolactin receptor and yet had normal gonadotropin secretion, a finding consistent with the previous observation that a deficit of prolactin seems to have no effect on gonadotropin secretion.

The inheritance pattern in the proband's family corresponds to an autosomal recessive trait, which suggests haplosufficiency of *PRLR* (i.e., having one fully functional allele of *PRLR* is sufficient to support lactation). Our biochemical data showed that both pathogenic variants imposed a loss of function on the receptor but did not have a robust dominant-negative effect on the nonvariant receptor. Both variants together led to profound loss of signal transduction. These data support our conclusion that biallelic loss-of-function variants in *PRLR* are the cause of the lactation phenotype in this case.

A short-loop, negative-feedback regulatory mechanism of prolactin on its own secretion has been shown in *Prlr* knockout mice.[16] Pituitary lactotrophs secrete prolactin that binds to the prolactin receptor of tuberoinfundibular dopaminergic neurons, which then produce dopamine. The released dopamine sequentially acts on pituitary lactotrophs and inhibits prolactin secretion.[17] Our data support the presence of this short-loop feedback regulation in humans.[16] Both mice and humans also show haplosufficiency of *PRLR* —

that is, a single functional copy of *PRLR* or (in mice) *Prlr* allele[18,19] is sufficient to maintain basal levels of serum prolactin within the normal range.

In a previous report,[3] heterozygotes carrying a loss-of-function variant (H212R) in *PRLR* had paradoxical symptoms (hyperprolactinemia and persistent postpartum galactorrhea). We found that the three variant receptors (R171Ter, P269L, and H212R) were almost similar in terms of residual signal-transduction function and the absence of a robust dominant-negative effect. Perhaps other factors that modulate prolactin receptor signaling explain the difference in phenotype between the two families.[2] Relevant to this hypothesis is the high level of variation in the relationship between serum prolactin levels and galactorrhea among women.[20] The results of our study involving a woman who was a compound heterozygote for loss-of-function mutations in *PRLR* and had agalactia associated with hyperprolactinemia would suggest that only lactation (and not other biologic functions) is dependent on prolactin–PRLR signaling, although further study is required in order to test this hypothesis.

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

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