Rescue of expression and signaling of human luteinizing hormone G protein-coupled receptor mutants with an allosterically binding small-molecule agonist

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Naturally occurring mutations of G protein-coupled receptors (GPCRs) causing misfolding and failure to traffic to the cell surface can result in disease states. Some small-molecule orthosteric ligands can rescue such misfolded receptors, presumably by facilitating their correct folding and shuttling to the plasma membrane. Here we show that a cell-permeant, allosterically binding small-molecule agonist (Org 42599) rescues the folding and cell surface expression, and therefore target cell signaling, of mutant human luteinizing hormone (LH) receptors (A593P and S616Y) that cause Leydig cell hypoplasia in man. Both mutant receptors were retained in the cytoplasm whereas WT receptor localized at the cell membrane, and binding of LH to cells expressing the mutant receptors was markedly lower than to those expressing the WT receptor. Incubation with Org 42599 increased mutant receptor expression, cell surface localization, and the proportion of mutant receptor in the mature glycosylated form. Importantly, although LH stimulated little (S616Y) or no (A593P) activation of cells expressing mutant receptors, incubation of cells with Org 42599 facilitated rescue of expression and stimulation by the native ligand, LH. Although Org 42599 could activate these receptors, it could not displace ¹²⁵I-labeled human LH binding to the WT receptor, indicating that it acts in an allosteric manner. Here we demonstrate a small-molecule GPCR allosteric agonist that functionally rescues intracellularly retained mutant LH receptors by facilitating their cell surface expression. This approach may have application for treatment of infertile patients bearing such mutations and, more broadly, for other misfolded GPCR mutants resulting in human pathologic processes.

pharmacological chaperone | receptor trafficking | infertility

Mutations in G protein-coupled receptors (GPCRs) have been identified for almost all endocrine hormone-signaling deficiencies. The luteinizing hormone (LH) receptor is a GPCR with a crucial role in reproduction. LH is secreted from the pituitary gland and is required for the induction of ovulation in females and for sex steroid hormone production in both males and females. Mutations in LH receptors can therefore lead to a spectrum of reproductive dysfunctions, with inactivating mutations leading to decreased sex steroid hormone production and/or problems with genitalia development (1–8).

Loss of function of mutant receptors can be a result of impaired ligand binding or coupling to intracellular signaling pathways, but most often are a result of poor cell surface expression of the receptors (9). The endoplasmic reticulum (ER) contains molecules involved in facilitating folding and transport of newly synthesized proteins, or in the recognition of misfolded proteins that are then targeted for degradation (10, 11). Many GPCRs have mutations that cause retention in the ER, resulting in disease states. Examples include mutations in the V₂ vasopressin receptor

that cause nephrogenic diabetes insipidus (12), mutations in rhodopsin that cause autosomal-dominant retinitis pigmentosa (13), and mutations in the gonadotropin releasing hormone receptor that cause hypogonadotropic hypogonadism (14, 15).

Some small-molecule orthosteric ligands (i.e., pharmacological chaperones) can interact with intracellularly retained receptors to aid their folding and transport to the plasma membrane. Examples include antagonists able to rescue cell surface expression of mutant V₂ vasopressin receptors (16), agonists and antagonists able to rescue mutant δ -opioid receptors (17), and an antagonist able to rescue mutant gonadotropin releasing hormone receptors (14). Such compounds therefore have important therapeutic potential.

In contrast to these orthosteric ligands, a group of thienopyrimidine compounds have recently been identified that allosterically activate the LH receptor (18). The present study demonstrates that one such thienopyrimidine, Org 42599 (Fig. S1), rescues plasma membrane expression and thereby signaling of two intracellularly retained mutant LH receptors (A593P and S616Y) identified in human patients with impaired reproductive function (for details of patient phenotypes, see *SI Text*). This compound has potential for use in the treatment of infertile patients bearing such LH receptor mutations and provides a unique therapeutic paradigm to use allosterically binding small-molecule agonists to target other misfolded GPCRs. The allosteric nature of the compound increases its therapeutic potential because, unlike previously described pharmacological chaperones, it does not compete with native ligands, thereby allowing activation of rescued mutant receptors by endogenous ligands.

Results

Mutant LH Receptors Are Retained Intracellularly. Cellular localization of myc-tagged WT and mutant LH receptors was determined by confocal microscopy by using an anti-myc antibody (green) and DAPI nuclear marker (blue). In the absence of ligand, the WT receptor was expressed at high levels and was predominantly located at the cell surface. However, both mutant receptors were expressed at much lower levels and were predominantly located

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Fig. 3. Org 42599 elicits robust activation of cells expressing WT and mutant LH receptors. Measurement of cAMP accumulation by CRE-luciferase reporter gene activation after 24 h stimulation in cells expressing WT (●), A593P mutant (■), or S616Y (▲) mutant LH receptors was determined over a range of concentrations of Org 42599 (*Materials and Methods*). Data were fitted by sigmoidal dose-response curves with Hill coefficients of unity. Data are presented as fold versus basal values for each receptor and are mean $\pm$ SEM from at least three independent experiments.

which resulted in large increases in cell surface receptor expression (intact cells) of the mutant receptors in a concentration-dependent manner (Fig. S5).

To determine whether the rescue of cell surface expression was a persistent or transient effect, cells were incubated with Org 42599 and the binding of 125 I-hLH measured directly, or after extensive washing out of the ligand for 18 h (which is necessary to remove all intracellular Org 42599). The increased binding of 125 I-hLH to cells expressing the mutant receptors after pre-incubation with Org 42599 was abolished after 18 h of washing, indicating that the rescue is transient (Fig. S6).

Incubation with Org 42599 Rescues Signaling in Cells Expressing Mutant LH Receptors. In contrast to LH, which stimulated little or no activation of cells expressing the mutant receptors in the CRE-luciferase reporter gene assay (Fig. 2B and Table S1), a large response to Org 42599 stimulation was observed (Fig. 3 and Table S1). This is presumably a result of concomitant receptor rescue and stimulation, as these experiments were conducted over a time frame of 24 h. Interestingly, the A593P mutant was less sensitive to stimulation by Org 42599 but revealed a greater efficacy. This may be a result of the dynamics of conformational instability and desensitization with the prolonged incubation necessary for rescue studies, which was not investigated further here.

To determine whether Org 42599 restored LH-stimulated signaling in cells expressing mutant receptors, cells expressing the S616Y mutant receptor were stimulated with LH after pre-

incubation in the presence or absence of Org 42599. Cells were preincubated with a lower concentration of Org 42599 (0.1 μ M) for shorter incubation times (2, 3, and 6 h) than used in the previous experiments to ensure that any cAMP accumulation induced by Org 42599 would be minimal, thus enabling the effects on the LH response to be determined more easily. In each case, a marked increase in cAMP response to LH in cells expressing the mutant LH receptor was seen for cells preincubated with Org 42599 (Fig. 5).

Discussion

The majority of loss-of-function GPCR mutations causing disease states are caused by misfolding and retention of the receptor in the ER and the subsequent lack of receptor at the cell membrane—its functional location. Cell-permeant orthosteric ligands that act as pharmacological chaperones can bind nascent receptor in the ER and rescue cell surface expression of these mutant receptors, and therefore have therapeutic potential (22). We now demonstrate that an allosteric LH receptor agonist is able to functionally rescue surface localization and also stimulate signaling of cells expressing two mutant LH receptors (A593P and S616Y) identified in patients with impaired reproductive function (1, 23). Rescue of surface expression with the allosteric agonist also increased signaling by the orthosteric endogenous ligand, LH.

Studies of 125 I-hLH binding with confocal microscopy visualization and Western immunoblotting of LH receptors demonstrated that A593P and S616Y mutant LH receptors are retained intracellularly in an immature form within the ER, in agreement with our previous study (24). As demonstrated herein and in the previous report (24), the decreased cell surface expression is more profound for A593P compared with S616Y. Moreover, the degree of intracellular retention and resulting decreased cell surface expression of the mutants correlates with the severity of the observed clinical phenotypes in the patients with these two LH receptor mutations (*SI Text* provides details of patient phenotypes) (1, 23, 25).

Although cell surface expression was impaired, neither receptor mutation affected the ability of LH to bind to the receptor as demonstrated in binding studies on cell surface and intracellular receptors (Figs. S2 and S3). This is not unexpected, as LH binding is located exclusively in the large N-terminal region of the receptor, and these mutations are both within the transmembrane domains. Although the mutant receptors have normal and high affinity for LH, cells expressing the mutants exhibited little (S616Y) or no (A593P) LH-stimulated cAMP response. This is a result of the decreased cell surface expression of the mutant receptors, thus leading to reduced agonist potency and maximal responses as described by the operational model of pharmaco-

Fig. 4. Binding of 125 I-hLH to cell-surface mutant LH receptors is increased in a time- and concentration- dependent manner after incubation with Org 42599. Binding of 125 I-hLH to cells expressing WT, A593P mutant, or S616Y mutant LH receptors was measured after incubation with Org 42599 (1 μ M) for a range of incubation times (A) or for 24 h with a range of concentrations of Org 42599 (B). After incubation with Org 42599, cells were washed once for 1 h with Complete media before incubation with radioligand. Data are presented as fold versus binding in the absence of Org 42599 treatment and are mean $\pm$ SEM from at least three independent experiments. Note WT receptor binding is not increased by Org 42599 treatment (1.0-fold vs. vehicle control), whereas mutant receptors show a time- and concentration- dependent increase in binding after incubation with Org 42599. * P < 0.05 and ** P < 0.01 (one-sample t test) for comparison with no change compared with vehicle control (1.0-fold change, dotted line).

Fig. 5. LH stimulation of cells expressing S616Y mutant LH receptors is increased by preincubation with Org 42599. cAMP accumulation was measured by cAMP ELISA after stimulation of cells expressing S616Y mutant LH receptors with LH (3 nM) for 1 h at 37 °C after preincubation in the presence or absence of Org 42599 (0.1 μM) for 2 to 6 h and washing once for 1 h (*SI Materials and Methods*). Data are mean ± SEM from three independent experiments and are presented as percentage of the maximum LH response obtained in the absence of Org 42599 incubation.

logical agonism (26). Decreasing WT LH receptor expression gives a similar decrease in potency and maximal stimulation of LH (27).

Incubation of cells expressing A593P and S616Y mutant receptors with Org 42599 increased the total level of mutant receptor expression, the proportion of mutant receptors in a mature form, and their cell surface expression. These data therefore suggest that Org 42599 facilitates the folding and transport of misfolded receptor retained within the ER, thereby increasing the trafficking of receptor protein to the cell surface and/or reducing the amount of receptor targeted to the proteasome. These mutant LH receptors have been shown to associate with different intracellular proteins compared with the WT LH receptor (24). Unlike the WT LH receptor, the mutant receptors interact with 94-kDa glucose-regulated protein (Grp94) and/or binding Ig protein, which are stress proteins that can aid protein folding and translocation within the ER but also have a role in transporting misfolded proteins to the proteasome. In addition, unlike the WT receptor, the A593P mutant receptor does not interact with protein disulphide isomerase, an enzyme involved in protein folding in the ER through catalysis of disulfide bonds (24). Thus, Org 42599 is presumably increasing association with some, and decreasing associations with other, intracellular proteins by binding to the receptors' allosteric sites to stabilize their folding within the ER. Further studies on the association of intracellularly retained mutant LH receptors with intracellular proteins in the absence and presence of Org 42599 should shed more light on these interactions.

In the CRE-luciferase reporter gene assay, Org 42599 was able to robustly activate cells expressing the mutant receptors whereas LH (without previous Org 42599 treatment) elicited little (S616Y) or no (A593P) response (Table S1). The CRE-luciferase reporter gene assay measures receptor activation after 24 h ligand incubation and therefore presumably allows for increased mutant receptor cell surface expression and concomitant or subsequent activation by Org 42599. Interestingly, the increased responsiveness to Org 42599 in the CRE-luciferase assay, after a long ligand incubation time (24 h), was not restricted to the mutant receptors. At the WT LH receptor, Org 42599 behaved as a partial agonist (achieving approximately 25% of the maximal response to LH) in the cAMP ELISA that entailed a 1 h incubation with ligand, but almost a full agonist in the CRE-luciferase reporter gene assay (Table S1). This may be a result of signal amplification in the CRE-luciferase assay and intrinsic limitations of the system or cells used preventing any further cAMP accumulation after activation with LH. The rescued functionality of the mutant receptors was further demonstrated by the increased response

to LH, measured in cells expressing these receptors, after preincubation with Org 42599 (Fig. 5).

Our findings suggest that Org 42599, or similar compounds, may be useful for treatment of infertile patients expressing these mutant receptors, and for whom there are currently no therapeutic options. Previous studies examining rat LH receptor mutants that are similarly misfolded and retained intracellularly demonstrated that incubations of cells at decreased temperatures rescued the mutants by increasing their cell surface expression (28). In agreement with the findings herein, it was observed that mutants with some degree of cell surface expression exhibited a greater ability to be rescued than those that were more profoundly retained (presumably as a result of more extensive misfolding). Although treatment with decreased temperature as a means to rescue misfolded LH receptor mutants provided a proof of principle regarding the potential restoration of target cell responsiveness by facilitating the folding and cell surface expression of otherwise intracellularly retained immature receptor, this method is obviously impractical from a therapeutic perspective. In contrast, it is entirely feasible that treatment with an allosteric agonist such as Org 42599 may be therapeutically relevant. It has previously been demonstrated that the closely related compound Org 43553 is orally active and able to induce ovulation in normal rodents (19), demonstrating the *in vivo* activity of this class of compounds and further supporting their clinical potential.

Both mutant receptors investigated in this study were rescued to a lesser or larger extent by Org 42599. However, it is not clear whether this would be the case for all ER-retained mutant human LH receptors. GPCRs represent 80% of all membrane receptors in man, and ER retention of GPCRs causes a wide range of disease states, so the use of pharmacological chaperones has vast therapeutic potential. As the compound described herein acts as an allosteric agonist, unlike previously described pharmacological chaperones, it has functions in rescuing cell surface expression, activating the mutant receptors, and allowing increased activation of the receptors with endogenous ligands. It thus has the capacity to facilitate responses to both Org 42599 and endogenous LH *in vivo*. Furthermore, mutations of GPCRs, including the LH receptor (6, 8, 29), have been identified in regions of the receptor that compromise binding of their cognate ligands. Therefore, there is potential for cell-permeant allosteric agonists such as the molecule described here to rescue the function of receptors with mutations affecting ligand binding.

In summary, this study identifies a low-molecular-weight allosteric agonist able to rescue cell surface expression, and therefore signaling, of two intracellularly retained, naturally occurring, mutant human LH receptors. This opens up the possibility of using such compounds for the therapeutic treatment of infertile patients with LH receptor mutations that cause intracellular retention of the misfolded receptors. Importantly, it provides a proof of concept for broader application in subjects with other mutant GPCRs.

Materials and Methods

Materials. Recombinant LH (lutropin alfa; Luveris) was obtained from Serono. Org 42599 was provided by Merck Sharp and Dohme. ¹²⁵I-hLH was iodinated in house by using hLH obtained from the National Hormone and Peptide Program, National Institute of Diabetes and Digestive and Kidney Diseases, and A. F. Parlow (National Hormone and Peptide Program, Torrance, CA).

Cell Culture. HEK 293 cells stably expressing WT or mutant human LH receptors with N-terminal myc tags were maintained in Complete media [DMEM, 10% FCS, 4 mM L-glutamine, penicillin (100 U/mL)/streptomycin (100 μg/mL)] supplemented with 500 μg/mL G418 when appropriate, at 37 °C in a humidified 5% CO₂ atmosphere. All cell culture plates and dishes were treated with Matrigel (BD Biosciences) before cell seeding unless mentioned otherwise. Cell culture reagents were obtained from Invitrogen or PAA Laboratories.

Confocal Microscopy. Cells, seeded in black poly-D-lysine-coated, glass-bottomed plates, were incubated with Org 42599 (1 μ M) or appropriate vehicle in Complete media for 24 h at 37 °C. Cells were then washed with PBS solution and fixed/permeabilized by incubation with methanol for 10 min at –20 °C. Cells were then washed three times with PBS solution (10 min each wash), followed by addition of blocking solution [PBS solution containing 20% normal goat serum (Biosera) and 2.5% BSA], and incubated for 1 h at room temperature. Following blocking, cells were incubated with primary antibody (mouse anti-c-myc clone 9E10; 1:50–1:100; Santa Cruz) overnight at 4 °C followed by three washes with PBS solution (10 min each wash), then incubated with secondary antibody (Alexa Fluor 488-conjugated goat anti-mouse; 1:200; Invitrogen) for 1 h at room temperature and washed three times with PBS solution (10 min each wash). Cells were then counterstained with DAPI (1:100; Sigma) for 5 min at room temperature before washing twice with PBS solution (5 min each wash). Finally, cells were visualized using an LSM 710 confocal microscope with a 40 $\times$ water-corrected objective (Zeiss).

cAMP Accumulation Assays. cAMP ELISA. Cells were incubated in the presence or absence of a range of concentrations of ligand (3 pM to 3 nM LH or 1 nM to 10 μ M Org 42599; LH concentrations calculated assuming a molecular weight of 28 kDa) in cAMP buffer (Hepes–DMEM supplemented with 0.1% BSA and 1 mM 3-isobutyl-1-methylxanthine) for 1 h at 37 °C. Cells were then lysed by incubation for 20 min with HCl (0.1 M). Cell debris was removed by centrifugation at 600 $\times$ g for 10 min, and the level of cAMP in the supernatants was determined using a direct cAMP enzyme immunoassay kit (Enzo Life Sciences). To determine activation of rescued receptors by LH, cells were preincubated with Org 42599 (0.1 μ M) in Complete media for 2 to 6 h and were then washed with Complete media for 1 h at 37 °C before incubation with LH (3 nM) in cAMP buffer for 1 h at 37 °C. Cells were lysed and cAMP levels measured as described earlier.

CRE-luciferase reporter gene assay. Cells were transiently transfected with Renilla luciferase (33 ng per well; Promega) and CRE-luciferase (467 ng per

well; Clontech) DNA using FuGENE 6 transfection reagent (Roche). At 24 h after transfection, cells were washed twice with PBS solution before addition of serum-free media [DMEM, penicillin (100 U/mL)/streptomycin (100 μ g/mL), 4 mM L-glutamine, 10 mM Hepes] and further incubation for 24 h at 37 °C. Cells were then incubated with a range of concentrations of ligand (3 pM to 3 nM LH or 1 nM to 10 μ M Org 42599; LH concentrations calculated assuming a molecular weight of 28 kDa) in serum-free media for 24 h at 37 °C. Cells were washed twice with PBS solution before lysis and measurement of luciferase activity using a Dual-Luciferase Reporter Assay System (Promega) and FLUOstar OPTIMA microplate reader (BMG Labtech). CRE-luciferase activity was normalized using the Renilla luciferase activity measurements for each well.

Data were calculated as fold versus basal for each receptor. Where appropriate, data were analyzed by nonlinear regression using GraphPad Prism software (GraphPad) and were fitted to sigmoidal dose–response equations to generate values for EC₅₀ and maximal stimulation.

Radioligand Cell Surface Binding Assays. Time and concentration dependence of receptor rescue. Cells were incubated for 1.5 to 36 h at 37 °C in the presence or absence of Org 42599 (1 μ M) in Complete media (for time dependence assay) or in the presence or absence of a range of concentrations of Org 42599 (10 μ M to 1 nM) in Complete media for 24 h at 37 °C (for concentration dependence assay). Cells were then washed with Complete media for 1 h at 37 °C before addition of [¹²⁵I]-hLH (100,000 cpm), prepared in binding buffer (Hepes–DMEM supplemented with 0.1% BSA), and incubated for 4 h at 4 °C. Cells were lysed by incubation for 15 min with NaOH (0.1 M), and their radioactivity was measured using a Wizard 1470 automatic γ -counter (Perkin-Elmer).

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- Kremer H, et al. (1995) Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet* 9:160–164.
- Misrahi M, et al. (1997) Comparison of immunocytochemical and molecular features with the phenotype in a case of incomplete male pseudohermaphroditism associated with a mutation of the luteinizing hormone receptor. *J Clin Endocrinol Metab* 82: 2159–2165.
- Leung MY, et al. (2006) Biological effect of a novel mutation in the third leucine-rich repeat of human luteinizing hormone receptor. *Mol Endocrinol* 20:2493–2503.
- Bruysters M, et al. (2008) A new LH receptor splice mutation responsible for male hypogonadism with subnormal sperm production in the propositus, and infertility with regular cycles in an affected sister. *Hum Reprod* 23:1917–1923.
- Huhtaniemi I, Alevizaki M. (2006) Gonadotrophin resistance. *Best Pract Res Clin Endocrinol Metab* 20:561–576.
- Huhtaniemi IT, Themmen AP (2005) Mutations in human gonadotropin and gonadotropin-receptor genes. *Endocrine* 26:207–217.
- Stavrou SS, et al. (1998) A novel mutation of the human luteinizing hormone receptor in 46XY and 46XX sisters. *J Clin Endocrinol Metab* 83:2091–2098.
- Huhtaniemi IT, Themmen APN (2000) Mutations of gonadotropins and gonadotropin receptors: Elucidating the physiology and pathophysiology of pituitary-gonadal function. *Endocr Rev* 21:551–583.
- Conn PM, Ulloa-Aguirre A, Ito J, Janovick JA (2007) G protein-coupled receptor trafficking in health and disease: Lessons learned to prepare for therapeutic mutant rescue in vivo. *Pharmacol Rev* 59:225–250.
- Ellgaard L, Helenius A. (2003) Quality control in the endoplasmic reticulum. *Nat Rev Mol Cell Biol* 4:181–191.
- Hebert DN, Molinari M (2007) In and out of the ER: Protein folding, quality control, degradation, and related human diseases. *Physiol Rev* 87:1377–1408.
- Morello JP, Bichet DG (2001) Nephrogenic diabetes insipidus. *Annu Rev Physiol* 63: 607–630.
- Noorwez SM, et al. (2003) Pharmacological chaperone-mediated in vivo folding and stabilization of the P23H-opsin mutant associated with autosomal dominant retinitis pigmentosa. *J Biol Chem* 278:14442–14450.
- Janovick JA, Maya-Nunez G, Conn PM (2002) Rescue of hypogonadotropic hypogonadism-causing and manufactured GnRH receptor mutants by a specific protein-folding template: Misrouted proteins as a novel disease etiology and therapeutic target. *J Clin Endocrinol Metab* 87:3255–3262.
- Millar RP, et al. (2004) Gonadotropin-releasing hormone receptors. *Endocr Rev* 25: 235–275.
- Morello JP, et al. (2000) Pharmacological chaperones rescue cell-surface expression and function of misfolded V2 vasopressin receptor mutants. *J Clin Invest* 105:887–895.
- Petäjä-Repo UE, et al. (2002) Ligands act as pharmacological chaperones and increase the efficiency of delta opioid receptor maturation. *EMBO J* 21:1628–1637.
- van Straten NC, et al. (2002) The first orally active low molecular weight agonists for the LH receptor: Thienopyrimidines with therapeutic potential for ovulation induction. *ChemBioChem* 3:1023–1026.
- van de Lagemat R, et al. (2009) Induction of ovulation by a potent, orally active, low molecular weight agonist (Org 43553) of the luteinizing hormone receptor. *Hum Reprod* 24:640–648.
- van Koppen CJ, et al. (2008) A signaling-selective, nanomolar potent allosteric low molecular weight agonist for the human luteinizing hormone receptor. *Naunyn Schmiedeberg Arch Pharmacol* 378:503–514.
- Finch AR, Sedgley KR, Caunt CJ, McArdle CA (2008) Plasma membrane expression of GnRH receptors: Regulation by antagonists in breast, prostate, and gonadotrope cell lines. *J Endocrinol* 196:353–367.
- Conn PM, Janovick JA (2009) Drug development and the cellular quality control system. *Trends Pharmacol Sci* 30:228–233.
- Latronico AC, et al. (1996) Brief report: Testicular and ovarian resistance to luteinizing hormone caused by inactivating mutations of the luteinizing hormone-receptor gene. *N Engl J Med* 334:507–512.
- Mizrachi D, Segaloff DL (2004) Intracellularly located misfolded glycoprotein hormone receptors associate with different chaperone proteins than their cognate wild-type receptors. *Mol Endocrinol* 18:1768–1777.
- Toledo SP, et al. (1996) An inactivating mutation of the luteinizing hormone receptor causes amenorrhea in a 46,XX female. *J Clin Endocrinol Metab* 81:3850–3854.
- Black JW, Leff P (1983) Operational models of pharmacological agonism. *Proc R Soc Lond B Biol Sci* 220:141–162.
- Bruysters M, Verhoef-Post M, Themmen AP (2008) Asp330 and Tyr331 in the C-terminal cysteine-rich region of the luteinizing hormone receptor are key residues in hormone-induced receptor activation. *J Biol Chem* 283:25821–25828.
- Jaquette J, Segaloff DL (1997) Temperature sensitivity of some mutants of the lutropin/choriogonadotropin receptor. *Endocrinology* 138:85–91.
- Qiao J, et al. (2009) A splice site mutation combined with a novel missense mutation of LHCR cause male pseudohermaphroditism. *Hum Mutat* 30:E855–E865.