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A Novel Mutation of the Human Luteinizing Hormone Receptor in 46XY and 46XX Sisters*

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ABSTRACT

We report a novel homozygous mutation of the LH receptor (LHR) gene in three siblings: two 46XY and one 46XX. The 46XY siblings presented with female external genitalia, primary amenorrhea, and lack of breast development. Hormonal evaluation revealed a markedly elevated LH level with a low testosterone level, which failed to increase after human CG stimulation. Enzymatic deficiencies of testosterone biosynthesis were eliminated as possible etiologies. Histologic analysis of the inguinal gonads in a 46XY sibling revealed no Leydig cells; Sertoli cells, spermatogonia, and primary spermatocytes were seen. The 46XX sibling had female external genitalia, normal breast development, and primary amenorrhea. Hormonal analyses showed markedly elevated LH levels and low plasma 17β-estradiol levels.

Genetic analysis of the LHR revealed a homozygous missense mutation at exon 11 of the LHR gene. Guanine was replaced by adenine (*G*AA→*A*AA), resulting in a substitution of lysine for glutamic acid (glu) at amino acid position 354 of the receptor. This mutation is located in the extracellular domain adjacent to the first transmembrane helix of the LHR. Glutamic acid at position 354 of the LHR has been highly conserved throughout evolution. Functional analysis of the LHR mutation, using an *in vitro* mutagenesis-transfection assay, demonstrated complete loss of function, indicated by the lack of cAMP production after human CG stimulation in transfected human embryonic kidney 293 cells. Screening of family members demonstrated heterozygosity for the mutation, indicating autosomal recessive inheritance. Delineation of the specific genetic defect in this family confirms recent reports that a single mutation in the LHR gene causes male pseudohermaphroditism in 46XY subjects and primary amenorrhea in 46XX subjects. More importantly, it also defines a new region of the LHR molecule that is critical for biologic activity. (*J Clin Endocrinol Metab* **83:** 2091–2098, 1998)

SEX determination in humans is genetically controlled (1, 2). Normally, 46XX individuals are phenotypically female and 46XY individuals are phenotypically male. In the presence of the SRY gene on the Y chromosome, the indifferent gonad develops as a testis (3–7). Antimullerian hormone and testosterone (T) (both secretory products of the testes) and dihydrotestosterone (DHT) (a peripheral conversion product of T) are required for the formation of normal male genitalia (8–11). Antimullerian hormone, which is synthesized by the Sertoli cells of the testes, inhibits the formation of Mullerian duct derivatives (uterus, fallopian tubes, and upper part of the vagina); whereas T, produced by the Leydig cells, stimulates differentiation of the Wolffian ducts to the vasa deferentia, epididymides, and seminal vesicles. DHT, a 5α- reduced product of T, acts locally to stimulate development of male external genitalia and prostate (12, 13). An abnormality in any of these processes, at a critical period *in utero*, will result in abnormal sexual differentiation.

In normal male sexual differentiation, placental human CG (hCG) binds to the LH receptor (LHR) of the Leydig cell, causing Leydig cell differentiation and resultant T production during the critical 8th–14th weeks of gestation (14–16). Failure of the LHR of Leydig cells to respond to hCG leads to a decrease or absence of T secretion and resultant male pseudohermaphrodism in 46XY individuals (17–25).

In contrast, normal ovarian differentiation and function are not required for normal female phenotypic development. However, during puberty and adulthood, LH stimulates ovarian thecal cells, via the LHR, to produce androgens, which are converted to estrogen by ovarian granulosa cells under the stimulation of FSH (26, 27). The crucial role of LHR seems to be in the mid-to-late follicular phase of the menstrual cycle, where it is acted upon by LH to promote follicular maturation and the development of a dominant follicle (28). The LH surge is mandatory for ovulation. In the luteal phase, LH stimulates corpus luteum formation and progesterone production.

In the present study, we report three sisters who were found to be homozygous for a unique LHR mutation. Two of the sisters (XY, XX) presented with primary amenorrhea. The 46XY sisters had external female genitalia with slight clitoromegaly, inguinal testes, and no breast development. The 46XX sister had a normal female phenotype and good breast development.

Case reports

Case 1. The 46XY proband is a 24-yr-old, phenotypic, and psychosexual female, who was referred for primary amen-

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orrhea and lack of breast development (Fig. 1, subject V-3). She was first evaluated at age 17. The subject is the offspring of a consanguineous marriage; her parents are second cousins (Fig. 1, subjects IV-3 and IV-27). She was the product of a normal spontaneous delivery, with no maternal history of gestational drug exposure. She is the eldest of eight siblings. Her mother was 14 yr old at the time of delivery. The subject had an uneventful childhood and experienced continued growth at age 15.

Physical examination at age 24 revealed a normotensive individual with a eunuchoid habitus (height, 170 cm; wt, 51 kg; arm span, 181 cm; head-pubis, 80 cm). Breast tissue was lacking. Genital examination revealed a Tanner stage 4 pubic hair, female external genitalia with a blind vagina pouch (4–5 cm long), slight clitoromegaly, and minimal posterior labial fusion. Axillary hair was diminished. The gonads were bilaterally palpable in the inguinal regions. Pelvic ultrasonography showed homogenous hypoechoic solid structures compatible with testes, measuring 3.2 cm × 1.2 cm × 2.2 cm in the right inguinal canal, 3.1 cm × 1.1 cm × 2.0 cm in the left inguinal canal, and normal kidneys. No uterus or ovaries were identified.

The subject underwent gonadectomy at age 24, and the inguinal testes were removed. Histology confirmed the absence of Leydig cells. Sertoli cells, spermatogonia, and primary spermatocytes were seen. Atrophic vasa deferentia and epididymides were present. Bone age was 13 yr, by the method of Greulich and Pyle (29).

Case 2. The fourth-born 46XY sibling was a phenotypic and psychosexual female (Fig. 1, subject V-7), who presented at age 10 with bilateral inguinal masses and slight clitoral enlargement. This sibling was the product of normal spontaneous delivery, without a history of drug exposure during pregnancy. She had female external genitalia with mild clitoromegaly, in addition to gonads that were bilaterally palpable in the inguinal regions. She died at age 20 of a malignant Schwannoma before genetic analysis of the LHR gene.

Case 3. The fifth-born sibling was 46XX (Fig. 1, subject V-8). She was 16 yr old at the time of evaluation and was a phenotypic and psychosexual female, who presented with primary amenorrhea. This subject was the product of normal spontaneous delivery, with no history of gestational drug

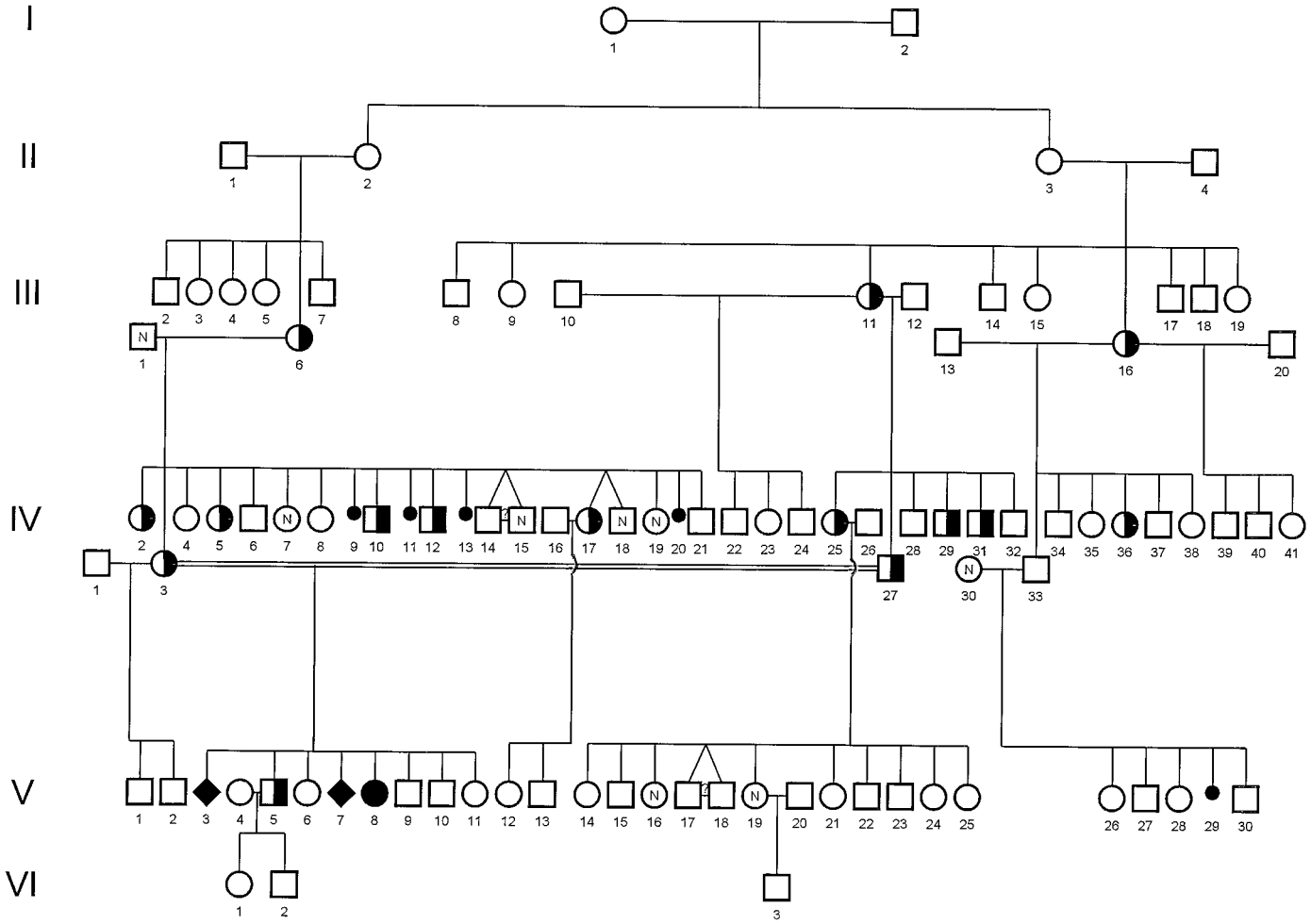

FIG. 1. Pedigree, demonstrating inheritance of an LHR mutation. *Circles* and *squares* indicate females and males, respectively. The *Roman numerals* indicate the generation. *Solid symbols* designate siblings with homozygous LHR gene defect and *hemi-solid symbols* designate those subjects with a heterozygous LHR gene defect. The *diamond shapes* designate 46XY female siblings.

exposure. She underwent thelarche at 9–10 yr of age and adrenarche at age 11–12. Her mother and maternal aunts experienced menarche at age 11. Her height was 151 cm., and her wt was 116 lbs (52.6 kg). Physical examination was notable for Tanner stage 5 breast development and Tanner stage 4 pubic hair. She had normal female external genitalia and decreased axillary hair. Transabdominal sonography revealed bilateral ovaries of normal size with evidence of stimulation, as suggested by the cystic appearance of the left ovary. A normal sized uterus was present.

Materials and Methods

hCG stimulation

hCG stimulation was initially performed on the 46XY sisters in 1989 using 1500 IU hCG, administered im three times per week for six doses. A second hCG stimulation test was performed on the proband in 1996 with 2000 U hCG (Pregnyl, Organon, Inc., West Orange, NJ) given im every other day for six doses. Plasma T, DHT, and Δ^4-androstenedione (Δ^4) levels were measured 24 h after the final dose of hCG.

Plasma hormones

Plasma T, DHT, and Δ4 were measured at baseline and after hCG stimulation, by RIA, after separation by paper chromatography, as previously described (30). Dehydroepiandrosterone sulfate was measured by RIA. Plasma estradiol concentrations were determined pre- and post hCG, by double antibody RIA (Diagnostic Product Corp., Los Angeles, CA) (31). Plasma LH and FSH concentrations were measured in duplicate by immunofluorometric assay, a highly sensitive, time-resolved immunofluorescence assay (Delfia Pharmacia-Wallac, Turku, Finland) (31).

Provera challenge

The 46XX sister was given 5 mg medroxyprogesterone acetate (Provera, Upjohn, Kalamazoo, MI) orally on days 1–12 of the calendar month.

PCR amplification and DNA sequencing

Blood was drawn into EDTA-containing tubes, and genomic DNA from white blood cells was isolated using a Qiagen genomic DNA isolation kit (Qiagen, Chatsworth, CA). The concentrations of DNA were determined by ultraviolet absorbance.

PCR amplification and labeling of exons 1–11 of the LHR gene were carried out using primers and conditions previously described (32), with minor modifications (available upon request). Five sets of inner primers were used for exon 11. The reaction mixture contained 0.12 μg or 0.36 μg genomic DNA, 200 μmol/L of each of four deoxyribonucleoside triphosphates, 1 μmol/L of each primer, 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L MgCl2, 0.1% Triton X-100, and 2.5 U thermostable DNA polymerase; 10 μCi [α-^{32}P]deoxy-ATP was added for the hot PCR. The samples were denatured at 94 C for 2 min and then sequentially denatured at 94 C for 30 sec, annealed at the temperature and duration previously described (32) with minor modifications, and extended at 72 C for 30 sec for a total of 35 cycles. A final extension cycle consisted of 72 C for 10 min. Sequencing of PCR-amplified products was performed with ^{32}P-end-labeled primer using an fmol DNA sequencing kit (Promega, Madison, WI).

Single-strand DNA conformational polymorphism (SSCP) analysis

SSCP analysis was performed as previously described (32a), with some modifications. Exon DNA was amplified and radiolabeled as described above. One microliter of the PCR product was added to 9 μL formamide denaturing dye (98% formamide, 20 mmol/L EDTA, 10 mmol/L NaOH, and 0.05% each of xylene cyanol and bromophenol blue) and then denatured at 100 C for 6 min and immediately cooled on ice. Three microliters of this solution were loaded onto a 0.5 × Hydrolink MDE gel (J. T. Baker Inc., Phillipsburg, NJ), containing 10% glycerol, and electrophoresed at 350 volts at room temperature overnight in 0.6 × TBE buffer (54 mmol/L Tris-borate, pH 8.3, and 2.4 mmol/L EDTA). An aliquot of hot PCR sample was diluted in sucrose loading buffer (60% sucrose, 50 mmol/L EDTA, and 0.05% each of xylene cyanol and bromophenol blue) and loaded in an adjacent lane, without denaturation, to determine the position of migration of the double-stranded DNA fragment. After electrophoresis, the gel was dried and exposed to Kodak X-OMAT film (Eastman Kodak, Rochester, NY) at room temperature.

Denaturing gradient gel electrophoresis (DGGE)

DGGE analysis of exon 11 of the LHR gene was performed using GC-clamp primers, as previously described (33). Briefly, fragments of exon 11 of the LHR gene were amplified by PCR using GC-clamp primers with thermal cycle conditions, as described above. Amplified exon DNA was denatured in 95 C for 10 min and reannealed by cooling down slowly to room temperature, and it was then electrophoresed in an 8% denaturing gradient polyacrylamide (19:1 acrylamide:bisacrylamide) gel in 0.5 × TBE buffer. The 100% denaturant was 7 mol/L urea plus formamide (60:40, by volume). Intermediate denaturants were prepared by diluting 100% denaturant with 8% acrylamide in 0.5 × TBE. Gradients were prepared bottom-up by gravity flow in a gradient maker. A Bio-Rad Miniprotein apparatus, with 1.5-mm spacers, was used for all of the experiments. The results were visualized by ethidium bromide staining.

In vitro mutagenesis and transfection studies

An expression vector (pCMX-LHR), containing the entire coding region of the human LHR gene, was kindly provided by Dr. A. J. W. Hsueh (Stanford University, Stanford, CA). Using *in vitro* mutagenesis (QuickChange Site-Directed Mutagenesis Kit from Stratagene, La Jolla, CA), a new subclone was constructed with a substitution of guanine by adenine at position 1060 of the human LHR gene. The sequences of all constructs were confirmed by DNA sequencing.

Human embryonic kidney 293 cells (ATCC, Rockville, MD) were grown in DMEM, supplemented with 10% FBS, 50 U/mL penicillin, and 50 μg/mL streptomycin. Cells (1.5×10^6) were plated in 60-mm dishes and transfected by the calcium phosphate precipitation method (ProFection, Promega, Madison, WI) with 10 μg expression vector, 2 μg RSV-β-galactosidase plasmid, and pBluescript-SK plasmid, to a total of 15 μg DNA/dish, as previously described (34). Sixteen hours after transfection, the cells were washed, and they continued to grow in fresh medium for 48 h until treated with hCG at concentrations from 10–1000 ng/mL. Two hours after hCG treatment, the cells were harvested, and cellular extracts were prepared for β-galactosidase activity measurements. The medium was collected for cAMP analysis, and the levels of cAMP were determined by RIA, according to the manufacturer's instructions (Biotrak c-AMPm ^{125}I assay system from Amersham, Arlington Heights, IL). The transfection efficiencies were monitored by measuring β-galactosidase activity in the cellular extracts, and the cAMP levels were normalized with transfection efficiencies.

Results

Hormonal evaluation

Hormonal evaluation of the 46XY proband at baseline, and after hCG stimulation, was performed at age 17 in 1989 and at age 24 in 1996, with similar results (Table 1). In both studies, plasma T and DHT levels were low, and they failed to increase after hCG stimulation (Table 1). The baseline level of LH was markedly elevated, with a normal-to-slightly elevated FSH level. Circulating levels of precursor steroids did not rise after hCG stimulation (Table 1).

The other (46XY) sibling was evaluated at age 10. Plasma T and DHT levels were low, and they failed to increase after

TABLE 1. Hormonal evaluation of 46XY (proband)

	LH (IU/L)	FSH (IU/L)	T (ng/dL)	DHT (ng/dL)	Δ4 (ng/dL)	DS (μg/dL)	17OHP (ng/dL)
3/2/88	38.6	7.2	6	1	2		66
1/18/89 baseline			15	3	11	51	112
1/25/89 1 week -hCG			13	4	11	37	129
2/1/89 2 weeks -hCG			9	3	12	52	136
2/8/89 3 weeks -hCG			15	4	14	67	129
10/1/96	31.1	11.82					
8/13/96 baseline			8	5	51	134	77
8/21/96 1 week -hCG			7	3	49	106	46
8/26/96 2 weeks -hCG			7	2	4	115	16
Normal baseline	1–9	1–10	266–1130	15–95	50–130	43–475	100–250

DS, Dehydroepiandrosterone sulfate; 17OHP, 17OH progesterone.

TABLE 2. Hormonal evaluation of 46XY sibling

	T (ng/dL)	DHT (ng/dL)	Δ4 (ng/dL)	DS (mcg/dL)	17OHP (ng/dL)
1/18/89 baseline	14	3	6	17	73
1/25/89 1 week -hCG	11	2	4	18	73
2/1/89 2 weeks -hCG	7	2	10	19	176
2/8/89 3 weeks -hCG	18	2	7	19	90
Normal (11–14 yr)	20–250	15–30	50–100	20–100	50–150

See Table 1 and text for the abbreviations.

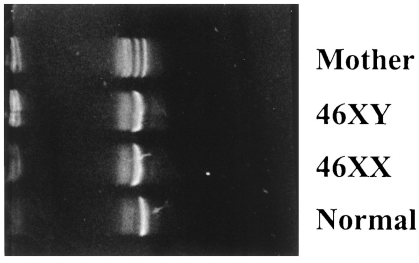

FIG. 2. A representative DGGE analysis of a PCR-amplified fragment of exon 11 of human LHR gene. Genomic DNA [from a normal control (lane 1), from the affected 46XX (subject V-8, lane 2), from 46XY proband (subject V-3, lane 3), and from their mother (subject IV-3, lane 4)] was extracted from the peripheral blood. A fragment of exon 11 of the LHR gene was amplified by PCR using GC-clamp LHR11N2-LHR11C2 pair primers and was analyzed with DGGE, as described in *Materials and Methods*. Clear differences in the band pattern and mobility were detected among these subjects.

hCG stimulation. In response to hCG stimulation, the levels of precursor steroids also did not increase (Table 2).

Hormonal evaluation of the 46XX sibling was performed every other day, for 1 month, and revealed an elevated LH and high-normal levels of FSH in the presence of low plasma levels of estradiol and progesterone throughout the month, with no cyclical changes. Basal body temperature measurements, performed every other morning, also did not support the occurrence of ovulation. All androgens measured were low (Table 3). During the second month of medroxyprogesterone acetate administration, the 46XX sister experienced vaginal bleeding.

The clinical and hormonal data from the three siblings were consistent with LHR unresponsiveness.

Genetic analysis

SSCP and/or DGGE was used to screen for a mutation of the LHR gene. As shown in Fig. 2, DGGE analysis of PCR-amplified products, located at the 5′ end of exon 11, revealed differences in the mobility of the bands between the normal control and the affected subjects. Heterozygosity was demonstrated also in the mother of the affected subjects. Similar changes were also observed by SSCP analysis (data not shown).

Using end-labeled primers, the PCR-amplified 5′ fragment of exon 11 of the LHR gene was sequenced for the proband (subject V-3), case 3 (subject V-8), their parents, and their grandmothers. A missense mutation in exon 11 of the LHR gene was identified (see Fig. 3). A guanine (G) is substituted by adenine (A) at position 1060 (the assignment starts at the translation start codon) of the nucleic acid sequence, causing the replacement of glutamic acid (Glu) by lysine (Lys) at amino acid position 354 (*G*AA→*A*AA) of the human LHR. This mutation is located in the extracellular domain adjacent to the transmembrane domain of the human LHR (Fig. 4). No other mutations were detected in the remainder of the coding regions of the LHR gene in the affected proband (data not shown).

Genetic analysis of the phenotypically normal mother (IV-3), father (IV-27), and grandmothers (III-6 and III-11) of the proband demonstrated heterozygosity for the same mutation (see Figs. 1 and 2). A phenotypically normal brother (V-5) was heterozygous for the same mutation and has fathered two phenotypically normal children. Several aunts and uncles of the proband were also found to be heterozygotes (Fig. 1).

In vitro mutagenesis-transfection analysis

The functional significance of this missense mutation was evaluated using *in vitro* mutagenesis transfection analysis in human embryonic kidney 293 cells, by measuring cAMP production, an immediate downstream marker of LH action. As shown in Fig. 5, hCG treatment, at doses of 10–1000 ng/mL, produced a dose-dependent increase in cAMP concentration in the medium of the wild-type LHR-transfected cells. At the highest hCG dose used (1000 ng/mL), the level

TABLE 3. Hormonal evaluation of 46XX sibling

	LH (IU/L)	FSH (IU/L)	Estradiol (pg/mL)	Progesterone (ng/dL)	T (ng/dL)	Δ4 (ng/dL)	17OHP (ng/dL)
Range	14–38	8–11	6–53	26–48	1–8	23–69	35–83
Mean ± SD	26 ± 9.2	9 ± 1.1	37 ± 11.8	37 ± 6.4	4 ± 2.3	43 ± 11	45 ± 13
Normal follicular	1–9	1–10	50–110	20–150	20–60	50–250	50–150
Postmenopausal	25–26	31–134	<30	<20	<25	<40	<50

The plasma levels of LH, FSH, estradiol, progesterone, T, Δ4, and 17OHP were measured every other day for 30 days. The values presented as the range and mean ± SD of the 15 samples. See Table 1 and text for the abbreviations.

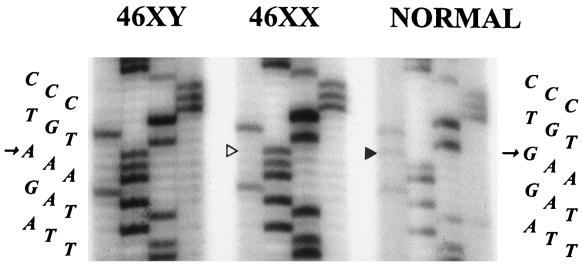

FIG. 3. A representative partial DNA sequencing of exon 11 of human LHR gene in normal control (*right panel*), 46XY (*left panel*), and 46XX (*middle panel*) patients. A PCR-amplified fragment of exon 11 of the LHR gene was sequenced as described in *Materials and Methods*. The *solid arrowhead* indicates that a guanine (G) in the normal sequence is substituted by an adenine (A), as indicated by an *opened arrowhead* in the affected subjects, resulting in a change of codon *G*AA to *A*AA.

of cAMP in wild-type LHR transfected cells was increased 12-fold, compared with vehicle control treatment. In contrast, the same hCG treatment, in the mutant LHR-transfected cells, failed to induce any cAMP production (indicating that the mutant LHR is essentially functionally crippled).

Discussion

We report a novel homozygous missense mutation of the LHR gene in 46XY and 46XX siblings. Additional phenotypically normal members of the family were genetically screened and found to be heterozygous for the mutation, including both parents, parental siblings, a male sibling, and both grandmothers (who were found to be first cousins) (Fig. 1). Thus, autosomal recessive inheritance was demonstrated by molecular genetic analysis and by pedigree analysis, confirming consanguinity.

The LHR gene mutation identified in this pedigree is a missense mutation located on exon 11 at position 1060 of the nucleic acid sequence, where an adenine (A) is substituted for a guanine (G), resulting in a change of codon 354 (*G*AA→*A*AA) of the LHR, from Glu to Lys (G354L). This mutation is located in the extracellular domain of the LHR, directly adjacent to the first transmembrane domain. It is different from previously reported homozygous LHR mutations (Fig. 4), which are located in transmembrane domains 5 and 6 (35–37) and in the extracellular domain (38) of the receptor, distant from the transmembrane domain.

Using *in vitro* mutagenesis-transfection analysis, the G354L mutation of the human LHR caused complete loss of receptor function, as indicated by the failure of cAMP production after hCG stimulation (Fig. 5). The substitution of an acidic amino acid, such as Glu^{354}, by a basic amino acid, Lys, could change the ionic environment and the conformation of the receptor, resulting in receptor inactivation (39). However, Thomas *et al.* (40) demonstrated that deletion of a large portion of the extracellular domain adjacent to the first transmembrane helix in the rat LHR did not alter the specificity and affinity of LH binding. More specifically, Huang and Puett (41) reported that this mutation of Glu to Lys in the rat LHR did not alter the binding affinity of LH, but it completely eliminated the hCG-stimulated cAMP production in transfected COS-7 cells.

Glu^{354} and the surrounding amino acids are a highly conserved region (see Fig. 6) in the human, rat, and porcine LHR (42–44); in the human, rat, monkey, and ovine FSH receptor (FSHR) (45–48); and in the human TSH receptor (TSHR) (49), suggesting a significant role in receptor function. Taken together, these results suggest that this highly conserved region participates in transmembrane signaling and has a fundamental role in signal transduction for all glycoprotein hormone receptors. Interestingly, all reported mutations causing inactivation of the human LHR are also located at highly conserved amino acids (35–37). Alternatively, a mutation of LHR (50), reported in a nonconserved amino acid, did not result in inactivation of the receptor.

The human LHR is a member of the G protein-coupled superfamily of receptors, characterized by the presence of seven transmembrane domains (42). Several mutations in the transmembrane domains of other receptors in this superfamily, which similarly cause loss of function, have been reported. These include inactivating mutations in the rhodopsin receptor (51), the vasopressin V2 receptor (52), the ACTH receptor (53), the thromboxane A2 receptor (54), and the calcium-sensing receptor (55).

Defects in the LHR result in abnormal Leydig cell differentiation and function at a critical period of sexual differentiation in affected 46XY subjects. Inadequate T and DHT production results in the development of abnormal male external genitalia. The phenotypes of 46XY subjects with LHR defects are variable. At one end of the spectrum are phenotypic males with micropenis and primary hypogonadism (36, 50), and at the other end are males with either female external genitalia or severe ambiguity of the genitalia (36, 37, present report). These phenotypic variations can be explained, at least in part, by the various defects of the LHR gene. It has been demonstrated that different mutations of the gene can cause either complete (37, present report) or partial (38, 50) loss of LHR function.

One interesting finding in male pseudohermaphrodites with LHR defects is the presence of atrophic epididymides and vasa deferentia. The differentiation and development of the epididymides and vas deferentia is dependent on androgens, as evidenced by studies of individuals with complete androgen-insensitivity (56). The presence of Wolffian duct structures in 46XY subjects with LHR defects suggests

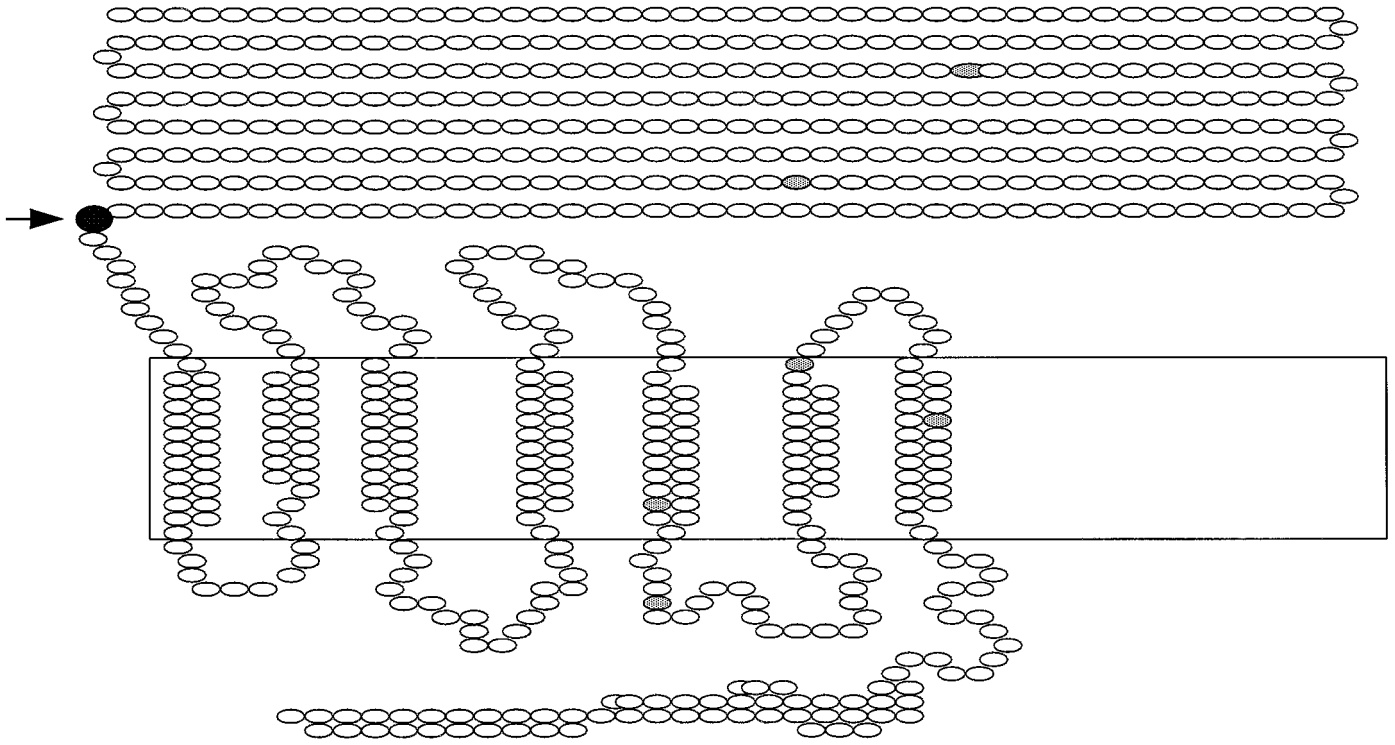

FIG. 4. A cartoon shows the overall structure of LHR with seven transmembrane domains separating three intracellular and three extracellular loops. The currently identified mutation (*solid symbol*) is located in the extracellular domain just adjacent to the first transmembrane domain, as indicated by an *arrow*, where a glu is replaced by an lys. Other reported mutations of human LHR are indicated by *dotted symbols*.

that a low level of T production is sufficient for Wolffian duct differentiation at the critical period *in utero*. T could be concentrated by androgen-binding protein secreted by the Sertoli cells in the seminiferous tubules, to promote the Wolffian duct differentiation (22). An alternative hypothesis, based on rabbit embryonic studies, is that the initiation of androgen synthesis at the beginning of male sexual differentiation is independent of extragonadal hormone stimulation (15).

The 46XX sibling with the G354L mutation in the LHR, reported herein, presented with primary amenorrhea and normal pubertal breast development. Analyses of her clinical and hormonal data suggest that LH action is necessary for complete follicular development and for ovulation (26–28). These findings are in agreement with previous reports of 46XX subjects with LHR mutations and similar phenotypes (36, 37). Histological analysis of an ovarian biopsy in one subject demonstrated morphologically normal primordial, preantral, and antral follicles (despite absent preovulatory follicles, corpora lutea, and corpora albicans) (37). Studies of porcine ovaries indicate that LHR-like immunoactivity and LHR messenger RNA are present in later stages of follicular development but not in primordial or primary follicles (28, 57). These results support the concept that initial follicular growth is independent of LH stimulation.

It is of interest that mutations of the LHR gene can also cause male limited autosomal dominant precocious puberty (58). In affected male subjects, the mutant LHRs are constitutively activated in the absence of ligand binding, resulting in excessive production of gonadal steroids and precocious puberty. All reported mutations of the LHR gene in subjects with precocious puberty are located in transmembrane do-

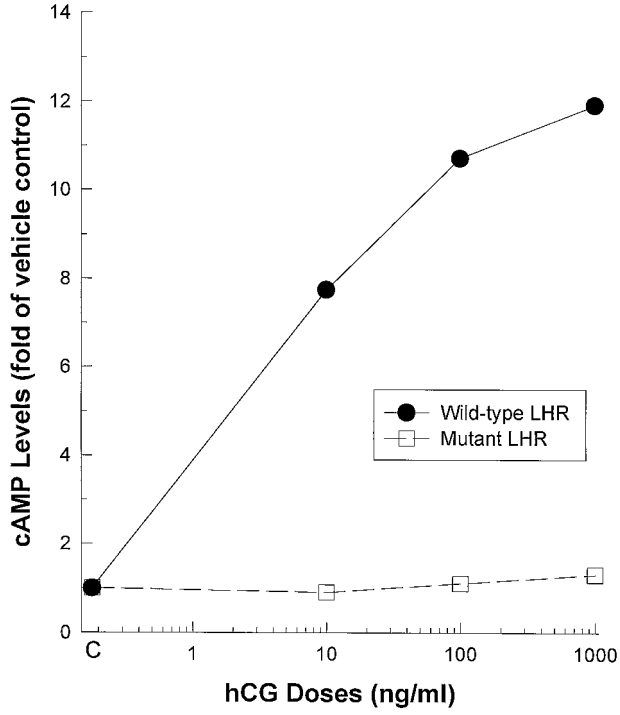

FIG. 5. Transfection analysis of the wild-type (*solid symbol*) and mutant (*open symbol*) LHR in 293 cells. An *in vitro* mutagenesis-transfection assay was performed, as described in *Materials and Methods*. cAMP concentration in the cell media was determined by RIA, 2 h after various doses of hCG treatment. The values are presented as folds of vehicle control treatment, and they are the mean of 3 experiments done in duplicate.

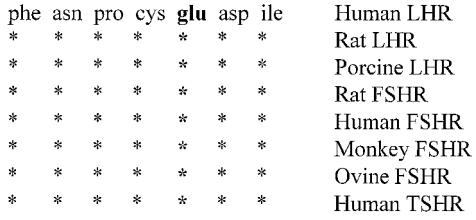

FIG. 6. The amino acid Glu354 is highly conserved through evolution. Partial amino acid sequences of human LHR, around Glu^{354}, is aligned with other related receptors; the conserved amino acids are indicated by an *asterisk*.

mains 5 and 6 (58), the same area involved in mutations causing inactivation of the LHR (35–37). However, in affected male subjects with an activated LHR gene mutation, 50% of the LHRs remain constitutively activated in the heterozygous state, leading to excessive production of gonadal sex steroids and precocious puberty.

In summary, a novel single-point mutation in the extracellular domain of the human LHR, adjacent to the first transmembrane domain, has been identified. This mutation causes complete inactivation of receptor function, resulting in male pseudohermaphroditism in the affected 46XY siblings and primary amenorrhea in the affected 46XX sibling of the same family.

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To honor Maria New for her lifetime achievements in pediatric endocrinology, a one day symposium will be held at the Villa Medicea "La Ferdinanda" in Artimino near Florence, Italy on 9/23–9/24/98. This conference will be held in conjunction with the annual meeting of the European Society for Pediatric Endocrinology in Florence.

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