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Neuroendocrine Phenotype Analysis in Five Patients with Isolated Hypogonadotropic Hypogonadism due to a L102P Inactivating Mutation of GPR54

Yardena Tenenbaum-Rakover, Monique Commenges-Ducos, André Iovane, Chantal Aumas, Osnat Admoni, and Nicolas de Roux

Pediatric Endocrine Unit (Y.T.-R., O.A.), Ha'Emek Medical Center, Afula 18101, Israel; Technion Faculty of Medicine (Y.T.-R.), 31905 Haifa, Israel; Maternité Pellegrin (M.C.-D.), Centre Hospitalier Universitaire Bordeaux, 33076 Bordeaux, France; Institut National de la Santé et de la Recherche Médicale U690, Hôpital Robert Debré (A.I., C.A., N.d.R.), 75019 Paris, France; and Université Paris 11 (N.d.R.), Faculté de Médecine Paris Sud, 94275 Le Kremlin Bicêtre, France

Context: Loss of function of the G protein-coupled receptor of kisspeptins (GPR54) was recently described as a new cause of isolated hypogonadotropic hypogonadism. *In vivo* studies performed in several species have confirmed the major role of kisspeptins in neuroendocrine regulation of the gonadotropic axis and therefore sexual maturation.

Objective: The objective of this study was to specify the exact contribution of kisspeptins and GPR54 to the initiation of puberty in humans.

Design: Detailed neuroendocrine descriptions were performed in five patients with isolated hypogonadotropic hypogonadism bearing a new GPR54-inactivating mutation.

Results: A homozygous mutation (T305C) leading to a leucine substitution with proline (L102P) was found in the five affected patients. This substitution completely inhibited GPR54 signaling. Phenotypic analysis revealed variable expressivity in the same family, either partial or complete gonadotropic deficiency. LH pulsatility analysis showed peaks with normal frequency but low amplitude. Repeated GnRH tests performed between 12 and 21 yr of age in one affected male revealed progressive changes in pituitary response from an early pubertal to an almost full pubertal pattern. Double GnRH test stimulations performed at a 120-min interval showed reduced dynamic pituitary response in GPR54-mutated patients.

Conclusion: GPR54 inactivation does not impede neuroendocrine onset of puberty; rather, it delays and slows down pubertal maturation of the gonadotropic axis. The L102P loss of function mutation in GPR54 results in a more quantitative than qualitative defect of gonadotropic axis activation. (***J Clin Endocrinol Metab* 92: 1137–1144, 2007**)

ISOLATED HYPOGONADOTROPIC hypogonadism (IHH) is defined as hypogonadism associated with low gonadotropin levels and normal levels of other pituitary hormones. IHH is divided into two groups based on the presence or absence of anosmia: association of IHH with anosmia defines Kallmann syndrome, whereas the absence of anosmia defines the idiopathic form of IHH. Loss of function mutations of the GnRH receptor accounts for 50% of the familial cases of idiopathic IHH (1). Recently, loss of function mutations of the kisspeptin receptor, also known as G protein-coupled receptor 54 (GPR54), have been suggested to account for some of the other 50% (2–5). Several reports have gone on to show the very high potency of kisspeptin in regulating LH and FSH secretion in many species (6–12), including humans (13). Although these findings represent a major breakthrough in our understanding of the physiology of the gonadotropic axis, the precise pathogenic mechanisms of impuberism resulting from GPR54 loss of function mutations remain unclear. GPR54 inactivation has been proposed to block pubertal onset (14). The advance of the vaginal opening in juvenile mice treated with kisspeptin (15) and the prepubertal increase of *KiSS-1* gene expression within the hypothalamus in nonhuman primates support the hypothesis that kisspeptins are involved in the resurgence of gonadotropic axis activation at puberty (8). The presence of micropenis and cryptorchidism in male infants bearing *GPR54* loss of function mutations has revealed that intact GPR54 function is also crucial for normal testosterone secretion in the fetus (5).

Although *GPR54* is expressed in both the hypothalamus and pituitary, *KiSS-1* is expressed only in the hypothalamus (16). GPR54 invalidation (GPR54 $-/-$) in mice does not change GnRH hypothalamic content and therefore has no effect on the migration or differentiation of GnRH neurons (3). It was thus suspected that kisspeptins may be involved in the regulation of GnRH secretion, and this was confirmed by using an *in vivo* approach in a sheep model (9) and rat hypothalamic explants (12). A stimulatory effect of kisspeptins on pituitary LH release was also shown using pituitary tissue (11).

In the last 3 yr, most of the knowledge on the physiological roles of GPR54 has been established from animal models. In humans, characterization of the phenotypes of affected patients with inactivating mutations of the *GPR54* gene is a

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Abbreviations: FAS, Free α-subunit; GPR54, G protein-coupled receptor of kisspeptins; hCG, human chorionic gonadotropin; IHH, isolated hypogonadotropic hypogonadism; IP, inositol phosphate; Kp10, 10-residue kisspeptin; L102P, proline substitution for leucine 102.

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relevant way to determine GPR54 function. In the present study, we identified a novel mutation in *GPR54*, a leucine substitution with proline at residue 102 (L102P), in five patients with IHH belonging to two unrelated Arab-Muslim families from Syria and Israel. We took advantage of a long follow-up of one patient bearing this L102P substitution to delineate the neuroendocrine phenotype and the consequences of GPR54 inactivation on pubertal maturation of the gonadotropic axis.

Patients and Methods

Family I

The proposita (patient I.1; Roman numerals indicate family number and Arab numerals indicate patients) was a female born from first cousin parents in Syria. She was referred for infertility at the age of 27 yr. She presented with primary amenorrhea and incomplete pubertal development (Tanner stage B4). She had no history of excessive exercise and no clinical signs of androgen excess. Her epiphyses were fused. Osteopenia of the femoral neck and lumbar spine was revealed by bone densitometry analysis (Z score −2.2 SD score). Evaluation of the hypothalamo-pituitary gonadal axis performed before administration of any estrogen and progestin treatment revealed low plasma estradiol (24 pmol/liter) with normal basal levels of LH and FSH (2.8 and 5.9 IU/liter, respectively). Plasma testosterone, androstenedione, dehydroepiandrosterone sulfate, prolactin, and cortisol levels were all within normal ranges. Pelvic ultrasonography revealed a small uterus (4.8 cm × 2 cm) with a thin endometrium (3 mm); ovaries were of normal size (23 × 15 mm and 24 × 14 mm) and contained five follicles in the right ovary and four follicles in the left, all smaller than 10 mm in diameter. One sister had primary amenorrhea at the age of 16 yr, and one brother presented with delayed puberty. The proposita was treated for 6 months with estrogen and progestin and then pulsatile GnRH (12 μg per pulse every 90 min) administration was initiated, which led to ovulation with luteal insufficiency. A second course of pulsatile GnRH administration led to ovulation on d 14 and to a normal pregnancy. After delivery, the proposita remained amenorrheic and was again treated with estrogen and progestin. Four years later, a second GnRH pulsatile treatment (10 μg per pulse) led to ovulation at d 20 and to a second pregnancy. Both children were born at term without any clinical anomalies. The placenta evaluated after the second pregnancy was normal. The proposita had no menstrual bleeding for several months after her second delivery.

Family II

The propositus was referred at the age of 10 yr for micropenis and bilateral cryptorchidism (Fig. 1A). He was born in Israel from Arab-Muslim consanguineous parents. Micropenis and cryptorchidism were noted at birth. At 12.5 yr, a hormonal study revealed low basal testosterone (0.1 ng/ml) and low LH and FSH levels (0.5 and 1.62 IU/liter, respectively). Right orchidopexy was performed at the age of 14 yr. From the age of 15.5 yr, he was treated with testosterone injections (50–250 mg monthly), except at 19 yr when testosterone therapy was replaced for 6 months with human chorionic gonadotropin (hCG) administration (2500 U im injection twice a week). Neither treatment induced significant changes in testicular volume, but some progress in pubic hair growth was observed. A pituitary adenoma of 10-mm diameter was demonstrated by brain magnetic resonance imaging, first at the age of 17 yr and in repeated imaging at 18, 20, and 21 yr without any change in size. Serum prolactin, GH, cortisol, and IGF-I levels were all within the normal ranges. At 21 yr, his height was 173 cm and he was overweight at 87 kg with a body mass index of 29 kg/m^2. He had pubic hair (P3), no facial hair, and a soft 3- to 4-ml testicular volume and 7.2-cm penile length. The propositus had 11 siblings: two sisters with primary amenorrhea (patients II.2 and II.3) and one sister with infertility for 5 yr (Fig. 1). Patient II.2 had incomplete pubertal development (Tanner stage P5B3

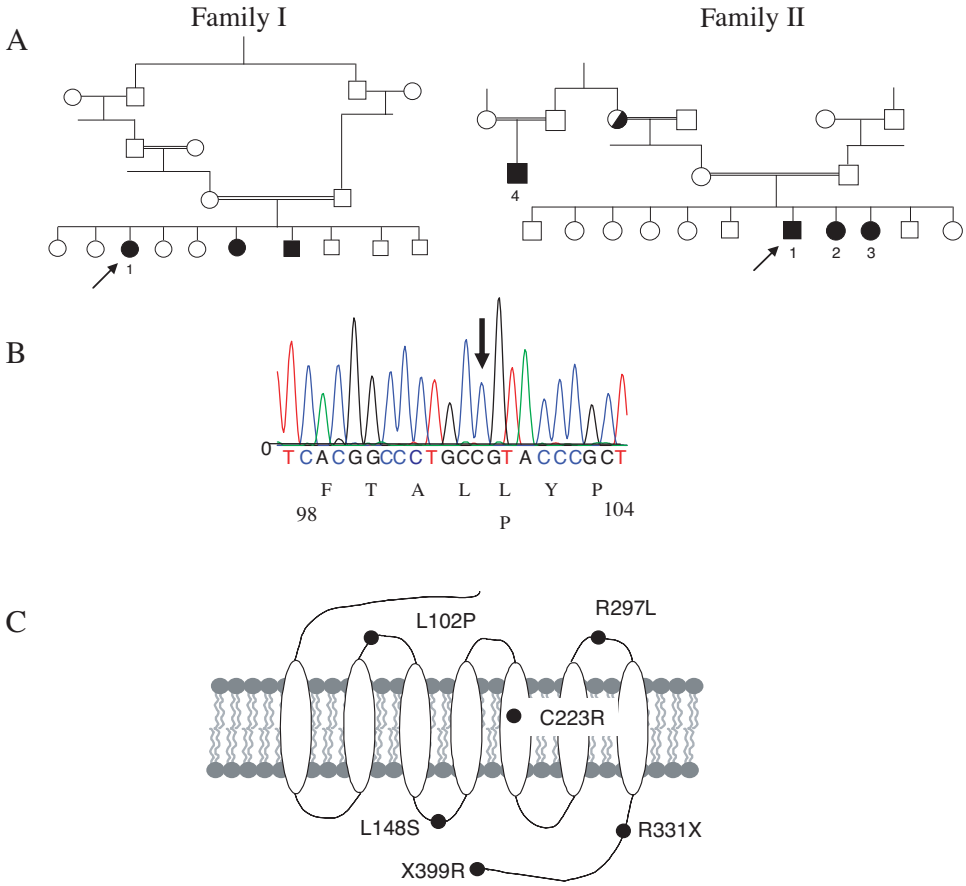

FIG. 1. Homozygote L102P mutation. A, Pedigrees of both families. Subject numbering is limited to the analyzed patients. For figure clarity, pedigrees were limited to four generations. *Arrows* indicate the probands. *Black symbols* indicate affected patients. B, Nucleotidic sequence of the PCR product amplified from DNA extracted from blood lymphocytes of one affected patient. The *arrow* indicates the mutated nucleotide. C, Localization of the L102P substitution and other published point mutations of GPR54.

at 17 yr) with low basal levels of LH (<0.5 IU/liter), FSH (1.1 IU/liter), and estradiol (<50 ng/ml). Pelvic ultrasound revealed a small uterus measured at 4.5 cm on the anteroposterior axis, and both ovaries were small with five 3-mm diameter follicles each. Patient II.3 was referred for evaluation of primary amenorrhea at the age of 16 yr. She had partial pubertal development (Tanner stage P3B3). Hormonal evaluation showed low basal levels of LH (1.3 IU/liter), FSH (4.0 IU/liter), and estradiol (<50 ng/ml). Pelvic ultrasonographic imaging showed a small 4.5-cm long uterus with a thin 2-mm endometrium; both ovaries were small. Treatment with a combination of estrogen and progesterone induced cyclic bleeding in both patients.

Patient II.4, a 32-yr-old man, was the uncle of patients II.1, II.2, and II.3, and he was born from first-cousin parents. He underwent investigation for infertility after 8 yr of marriage. Bilateral orchidopexy was performed at the age of 16 yr. He had 3-ml testes, P5 stage pubic hair, a penile length of 5 cm, low LH and FSH levels (<0.5 IU/liter and <0.4 IU/liter, respectively), and low basal testosterone concentration (0.34 ng/ml). He was treated with monthly testosterone injections. Sperm count and testicular biopsy revealed azoospermia.

Analysis of gonadotropin secretion

In the proposita of family I, a GnRH test was performed before initiation of any treatment, at 25 yr. In the propositus of family II, GnRH tests were first performed at 12.5 yr and were repeated at the ages of 16.75, 18, 19.75, and 21.6 yr. In the affected patients of family II, two GnRH (100 μg iv) stimulations were performed at a 120-min interval to evaluate pituitary GnRH priming (17, 18). LH and FSH levels were measured every 15 min over a period of 240 min. Although commercial methods for LH and FSH measurements during GnRH tests have changed over the years, references for normal values remain similar. Pulsatile LH, FSH, and free α-subunit (FAS) secretions were evaluated in the proband of family I by 10-min sampling over a 6-h period 24 months after the first pregnancy. Pulses were analyzed by Cluster8 from PulseXp software (19). Plasma LH, FSH, and FAS concentrations were measured by commercial methods (Immunotech, Marseille, France; and Bayer Diagnostics, Tarrytown, NY). For all assays, the intra- and interassay coefficients of variation were less than 5%.

DNA sequencing

After informed written consent was obtained, genomic DNA was extracted from peripheral blood mononuclear cells of the affected individuals and used for sequencing GPR54 exons (2).

Construction of the expression vector

Human GPR54 cDNA was cloned by RT-PCR from human placenta. It was subcloned into pcDNA3.1(−) plasmid at the *Eco*R1 site. The L102P mutation was introduced into the vector encoding the wild-type receptor by PCR method. Plasmid construct was validated by dideoxy sequencing of the modified fragment and restriction-enzyme mapping.

Transfection and functional studies

Accumulation of inositol phosphate (IP) was measured 48 h after transfection of the plasmid encoding the wild-type receptor or the L102P-mutated receptor in HEK293 cells. Transient transfection with 1 μg of vector DNA was performed after 24 h by using lipofectin plus (Invitrogen, Cergy-Pontoise, France). Twenty-four hours after transfection, culture medium was replaced with inositol-free DMEM supplemented with labeled inositol (2 μCi/well myo-[^{3}H]inositol; GE Healthcare Europe, Orsay, France). Twenty-four hours later, cells were incubated with 10 mM LiCl for 10 min and then stimulated with a 10-residue kisspeptin (Kp10) for 30 min at 37 C (Phoenix Pharmaceutical, Belmont, CA). The natural ligand of GPR54 is a 54-residue kisspeptin, which has been purified from placenta (16). Kp10, which is a decapeptide corresponding to the most C-terminal portion of 54-residue kisspeptin, stimulates GPR54 with a similar potency (16). IP was then purified as previously described elsewhere (20).

Results

Sequence analysis of the GPR54 coding sequence

Affected patients were all born from consanguineous parents (Fig. 1A). Sequencing of the five exons of the *GPR54* gene revealed one homozygous mutation in exon 2 in all affected patients from family I and family II (Fig. 1B). A substitution of a cytosine for thymidine 305 yielded a proline substitution for leucine 102 (L102P). This residue is localized within the first exoloop (Fig. 1C). Nonaffected patients who were available for analysis showed the T305C heterozygous mutation or the normal sequence. In exon 5, a heterozygous substitution of an adenine for a thymidine at position 1091 changes the codon CAC for CTC and results in a L364W substitution. Sequenced DNA samples from our cohort of hypogonotropic hypogonadism cases showed a frequency of 64% for the allele CAC and 36% for the allele CTC as previously reported in a control population (4).

Functional characterization of the mutated L102P receptor

The L102P-mutated receptor was transiently expressed in HEK293 cells and challenged with stimulation by Kp10 (Fig. 2). GPR54 stimulates PLC activity via protein Gq. Intracellular accumulation of IP was measured after incubation with 10^{-6} M Kp10 for 30 min. Virtually no IP accumulation occurred on Kp10 stimulation of cells expressing L102P mutated receptor compared with the wild-type receptor (Fig. 2A). No increase in IP accumulation was observed for HEK293 cells transfected with pcDNA3.1 plasmid alone.

The L102P-mutated receptor's affinity for Kp10 was studied by competitive binding for ^{125}I-Kp10 by unlabeled Kp10 on intact cells. The binding affinity (Ki) of the wild-type receptor for Kp10 was calculated at 0.6×10^{-9} M. A similar binding affinity was calculated for the L102P-mutated receptor (1.1×10^{-9} M). A decrease in cell-surface binding sites was observed in HEK293 expressing L102P receptor when compared with the wild-type receptor (Fig. 2B).

Pulsatile LH secretion analysis

In the proband of family I, analysis of LH and FSH pulsatility was performed over a period of 6 h after release of any treatment and 24 months after the first pregnancy (Fig. 3). Analysis of LH pulsatility revealed low peak amplitude, whereas the frequency of those peaks was very homogeneous. Analysis of LH plasma levels with Cluster8 from PulseXp software revealed six significant peaks over a period of 6 h. A similar decrease in peak amplitude was observed for FSH, although no significant peaks were observed. Analysis of FAS pulsatility revealed several peaks with low amplitude, but these were nonsignificant.

GnRH test analysis

In the proband from family I, a GnRH test performed before the first pregnancy showed a positive LH response and a persistent FSH response at 90 min (Fig. 4). In family II, GnRH tests performed at 16 yr showed intrafamilial heterogeneous LH and FSH responses. Administration of GnRH (100 μg iv) resulted in a low LH increase in the proband of family II, a high and quick LH increase in his sister II.3,

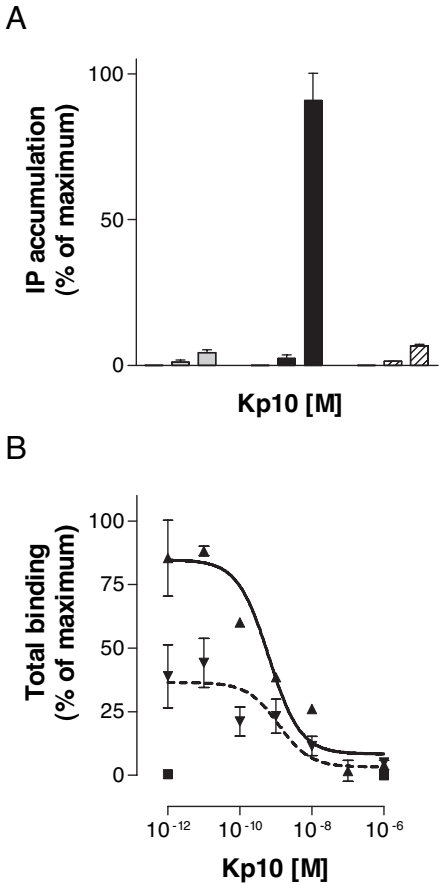

FIG. 2. L102P mutation inactivates GPR54 signal transduction under Kp10 stimulation. A, Kp10-stimulated generation of IP measured 48 h after transfection (*closed bars*, wild-type receptor; *hatched bars*, L102P receptor; *gray bars*, pcDNA3.1). Kp10 10^{-13} M was compared with 10^{-6} M. B, 125I-kisspeptin binding on HEK293 cell surfaces. HEK293 cells were transiently transfected with wild-type GPR54 (▲), L102P-mutated GPR54 (▼), or pcDNA3.1 (■). Presented data are values from one of three representative experiments. They are given as percentage of the maximum values observed for the wild-type receptor.

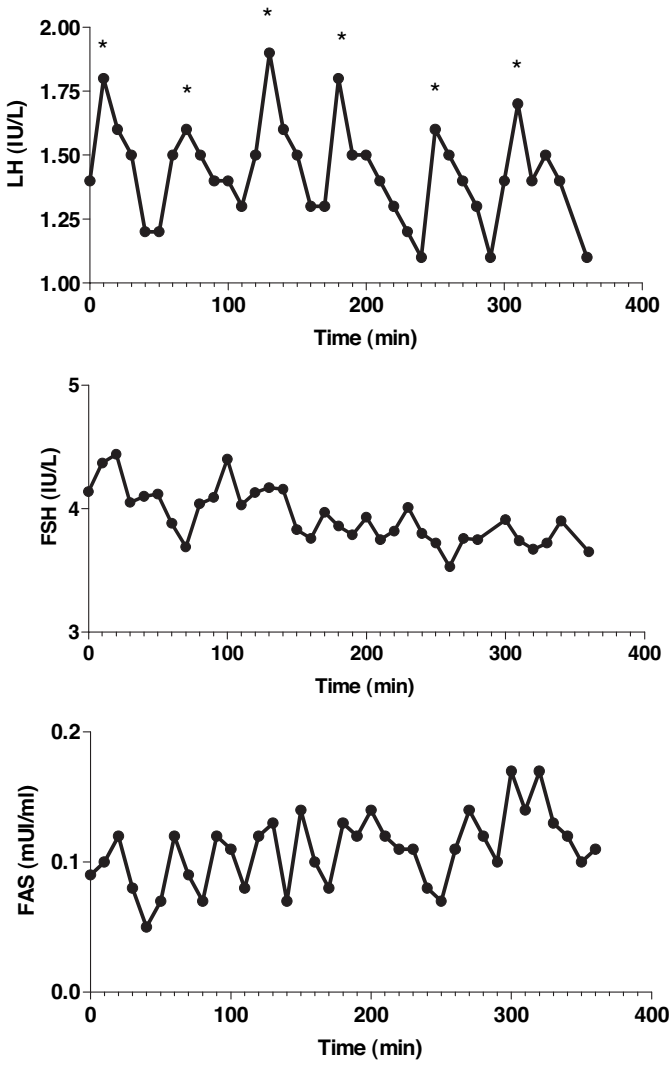

FIG. 3. Pulsatile secretion of LH, FSH, and FAS in the proband of family I. Blood was collected every 10 min for 6 h. LH, FSH, and FAS were measured with highly sensitive kits as described in *Patients and Methods*. Note the different scales among LH, FSH, and FAS. Peaks were analyzed by PulseXp software. *, Significant LH peaks.

whereas a blunted response was observed in patient II.2. Low FSH responses to GnRH stimulation were observed in patients II.1 and II.3 of family II but no response was observed in patient II.2 (Fig. 4).

In the proband of family II, six GnRH tests were performed over a period of 10 yr. The basal LH concentration increased from 0.50 IU/liter at 12.5 yr to 1.7 IU/liter at 21.6 yr. The patterns of the LH response to GnRH stimulation showed two remarkable differences. The maximum increase in LH peak rose from 1.98 IU/liter at 12.5 yr to 5.5 IU/liter at 21.6 yr, and the maximum increase occurred earlier (25 min) at 21.6 yr compared with 12.5 yr (45 min) (Fig. 5A). We next calculated the LH/FSH ratio, which is considered a good indicator of pubertal maturation of the pituitary when it is above 1. Basal and stimulated FSH levels were higher than LH concentrations in the tests performed at 12.5, 15, 16.75, and 18 yr (Fig. 5B). Although basal LH/FSH ratios were 0.65 and 0.97, GnRH-stimulated LH/FSH ratios were all above 1 in the tests performed at 19.75 and 21.6 yr.

To complete the functional characterization of the pituitary response to GnRH stimulation, we performed a priming test with repeated GnRH stimulations at a 120-min interval in patients II.1, II.2, and II.3 of family II. A 2-fold increase in the GnRH-induced LH peak was expected after the second GnRH stimulation (17, 18). A nonsignificantly higher LH peak was observed after the second GnRH administration in patient II.3 (Fig. 6). In patient II.2, no LH increase was observed after the first GnRH administration, whereas a low rise in LH levels was observed after the second GnRH stimulation. There was no difference between these peaks in patient II.1. Continuous increases in FSH concentration were observed after both GnRH stimulations for patients II.1, II.2, and II.3 from family II (Fig. 6).

Discussion

GPR54 and kisspeptin play a major role in neuroendocrine regulation of the gonadotropic axis. Although this novel

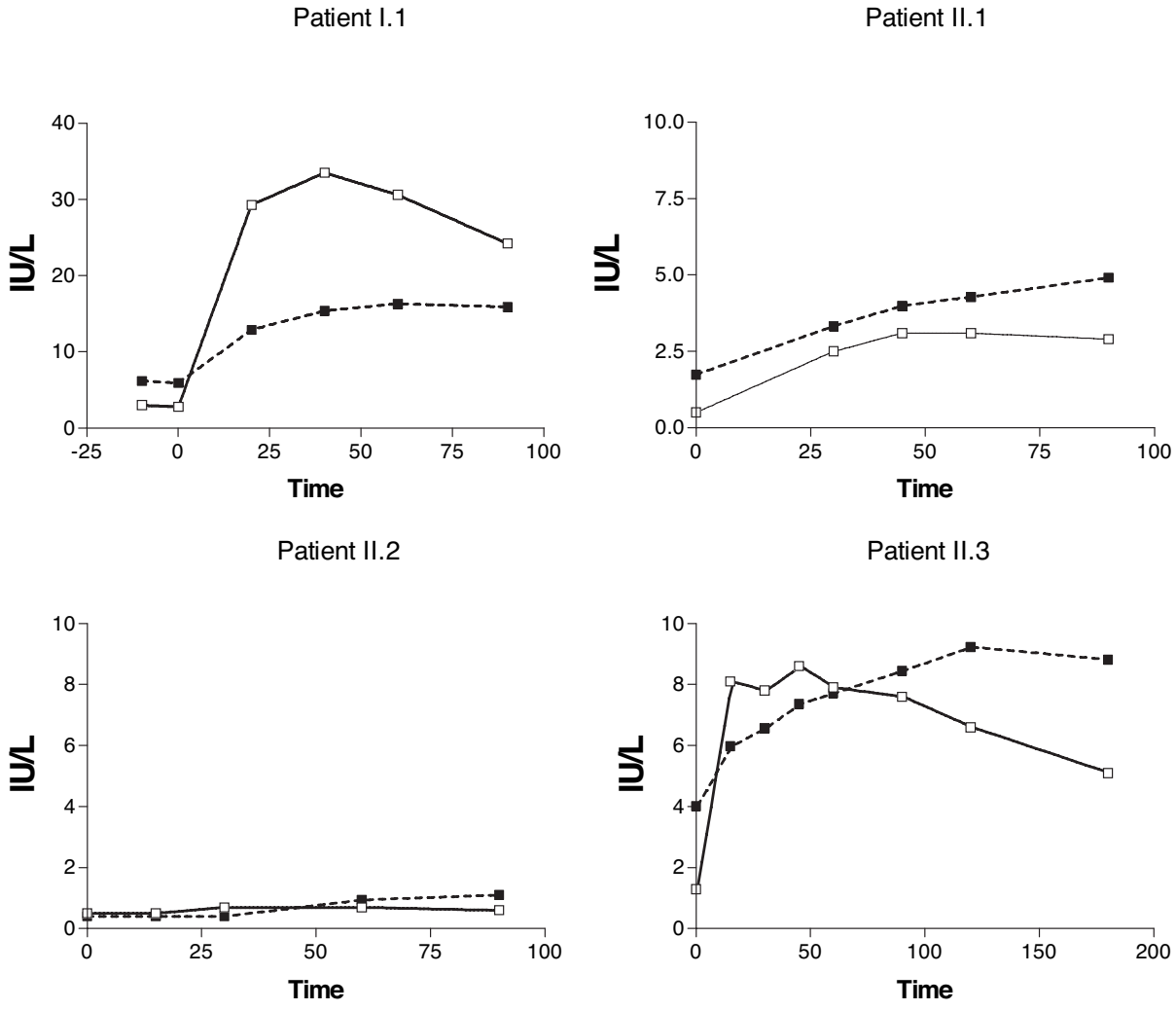

FIG. 4. GnRH tests performed in families I and II. GnRH (100 μg) was administered iv at distance from any hormonal treatment. For patient I.1, the GnRH test was performed at 25 yr. For patients from family II, GnRH tests were performed at 16 yr. □, LH levels; ■, FSH levels.

concept was first established by human genome mapping in IHH familial cases (2, 3), only two additional clinical descriptions of IHH cases resulting from GPR54 inactivation have been reported to date (4, 5). To increase our knowledge of this new neuroendocrine system in the physiology of pubertal onset in humans, we performed a detailed description of the neuroendocrine phenotype in five patients harboring a loss of function mutation of GPR54. Our results suggest that the impuberism observed in these patients results mainly from a quantitative alteration of gonadotropic-axis activation.

Phenotypic analysis of the proband of family I revealed persistent pulsatile secretion of LH with normal frequency but low amplitude when compared with normal values in the follicular phase (21). Gonadotropic-axis activation is a dynamic process that first occurs during fetal life. After its inhibition during childhood, a resurgence of gonadotropic axis activation occurs in the prepubertal period before the appearance of pubertal signs (22). In humans, nocturnal LH peaks with persistent, very low diurnal LH levels represent the earliest measurable event of puberty. As puberty progresses, LH and FSH pulse frequencies and amplitudes become more homogeneous. These changes in LH and FSH plasma levels have been attributed in nonhuman primates to an increase in GnRH secretion within the pituitary portal system (22). The LH peak frequency of one peak per hour observed in the proband of family I was similar to the frequency observed in the mid and late follicular phase in normal adult women (21, 23). GnRH pulsatility is an intrinsic property of GnRH neurons (24–27). It has been proposed that a specific network leads to concomitant release of GnRH from each GnRH neuron (28). The persistent homogeneous pulsatile secretion of GnRH revealed by the LH pulsatility analysis indicates that GPR54 is not directly involved in the synchronization of this hypothalamic network. GPR54 inactivation in humans does not block normal differentiation and prepubertal maturation of the GnRH hypothalamic neurons.

In humans, a positive GnRH test attests to GnRH pituitary priming and therefore initiation of puberty. Differences in the patterns of the GnRH test throughout normal puberty have been described. In the proband of family II, analysis of the GnRH test over a period of 10 yr also showed some

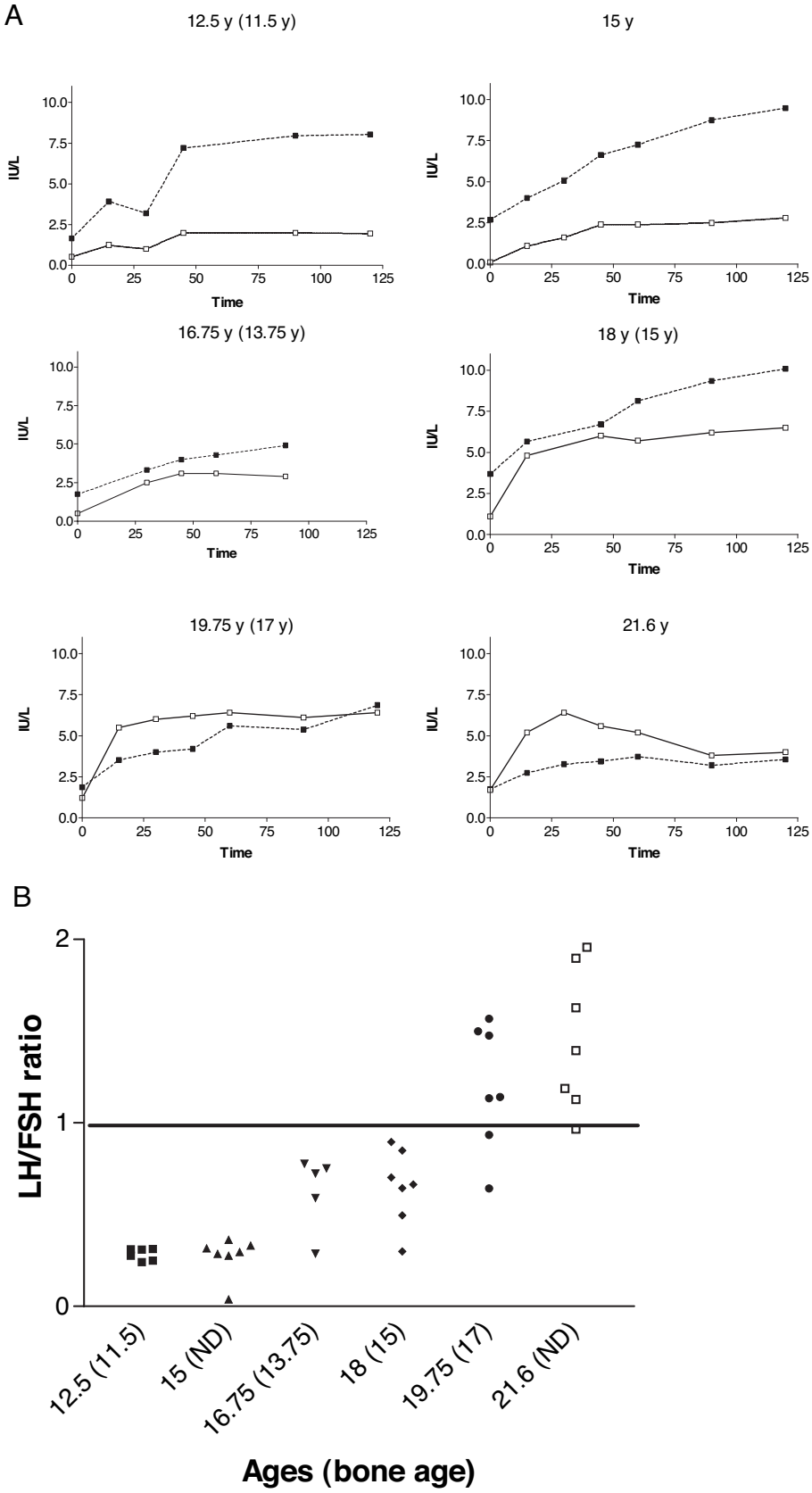

FIG. 5. A, GnRH tests were performed over a period of 10 yr on the proband of family II. For each test, chronological age is indicated with bone age in *brackets* when available. □, LH levels; ■, FSH levels. B, Evolution of the LH/FSH ratios at each time point over a period of 10 yr in the proband of family II. ND, Not determined.

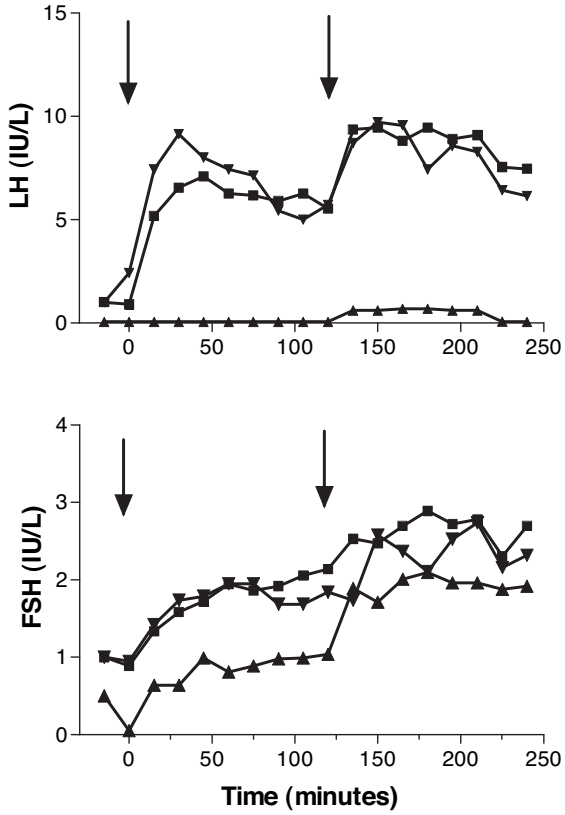

FIG. 6. Double GnRH stimulations in affected patients of family II. *Arrows* indicate iv GnRH (100 μg) administration. ▼, Patient II.1 (22 yr); ▲, patient II.2 (19 yr); ■, patient II.3 (18 yr).

variations. The GnRH-induced LH and FSH response observed in this patient at 18 yr with LH/FSH ratios less than 1 was similar to those observed in mid-puberty, whereas the responses at 19.75 and 21.6 yr were similar to the profiles described in late puberty, albeit with lower LH peak increases (29). It is interesting to note that LH levels increased from 12.5 yr (2.2 IU/liter) to 21.6 yr (5.5 IU/liter), although LH/FSH ratio inversions may also have resulted in part from a lower increase in FSH levels to GnRH stimulation at 19.75 and 21.6 yr relative to 18 yr. Such a decrease in the GnRH-induced FSH rise has been previously observed in normal puberty. Together, these results show that pituitary GnRH priming occurred with a large delay in patient II.1 and that it followed a normal process, albeit at a slower pace as it occurred over a period of 7 yr. This slow GnRH pituitary priming might be related to the low hypothalamic GnRH release in these GPR54-mutated patients.

The present study shows that the same mutation of GPR54 may lead to normal as well as completely blunted LH and FSH responses to GnRH stimulation. The fact that the pituitary response to GnRH stimulation is multifactorial was already suggested when variable GnRH-induced LH and FSH releases were reported for the same GnRH receptor mutation (30, 31). Because GPR54 is expressed within the pituitary, it might be one of the cofactors of the GnRH receptor signaling pathway. However, factors other than GPR54 activation seem to be involved in this modulation. The absence of a further increase in LH release in response to the second GnRH stimulation in the double-GnRH-stimulation protocol is another argument suggesting that GPR54 may have a direct effect on pituitary function. The hypothesis that kisspeptins, through GPR54 activation, are positive modulators of GnRH-induced LH release is under investigation in our laboratory. This modulation may take place at puberty, but also during the GnRH-induced LH ovulatory peak in women.

Analysis of FAS pulsatility is another way of delineating pituitary gonadotropic function in GPR54-mutated patients. In fact, concomitant secretion of FAS and LH has been observed *in vivo* (32) as well as in perifused pituitaries (33). Moreover, continuous treatment with GnRH agonist decreases LH secretion but increases plasma FAS levels (34). FAS-pulsatility analysis in the proband of family I showed concomitant secretion with LH and additional peaks. Similar higher FAS pulse frequencies have been reported in normal women (35), suggesting a possible dissociation between pituitary secretion of FAS and LH. FAS uses a specific secretory pathway, which is not directly regulated by GnRH and does not appear to involve GPR54.

The low testicular response to hCG therapy in patient II.1 from family II shows the low efficiency of this approach in GPR54-mutated patients as previously reported in a compound heterozygote patient for two point mutations in GPR54 (5). In contrast, GnRH pulsatile treatment increased plasma gonadotropin levels, leading to normal testosterone levels and oligoasthenozoospermia in a patient bearing a homozygous 1001–1002insC insertion of GPR54 (4). This difference may be related to the use of GnRH agonist rather than hCG treatment without FSH or to the potentially lower functional defect caused by the 1001–1002insC compared with the L102H substitution. It may also be related to testicular damage resulting from delayed orchidopexy.

Functional analysis of the L102P-mutated receptor confirmed that the PLC pathway is the GPR54 signaling pathway involved in gonadotropic axis regulation as previously suggested in other studies (2, 3, 5). The absence of IP accumulation under Kp10 challenge in cells expressing the L102P-mutated receptor shows that the L102P substitution completely blocks the capacity of GPR54 to activate the PLC pathway. Proline substitution of a hydrophobic residue such as leucine induces a major conformational change in the receptor. Cell-surface binding analysis revealed normal affinity of the L102P receptor for Kp10 and a small decrease in cell-surface expression. These results indicate that this amino acid substitution within the first extracellular loop blocks the normal conformational change of the receptor during activation as proposed for other G protein-coupled receptors (36). This functional analysis shows that a complete defect in GPR54 receptor signaling results in partial gonadotropic deficiency. Mechanisms governing the resurgence of gonadotropic-axis activation at puberty are thus not restricted to GPR54 activation.

These detailed phenotypic analyses of patients bearing GPR54 mutations show that GPR54, and therefore kisspeptins, must be considered modulators of GnRH action during fetal life, at puberty, and in adulthood. The gonadotropic deficiency observed in GPR54-mutated patients results in a

more quantitative than qualitative defect in the pubertal process.

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Address all correspondence and requests for reprints to: Dr. Nicolas de Roux, Institut National de la Santé et de la Recherche Médicale U690, Hopital Robert Debré, 48 Bd Serrurier, 75735 Paris Cedex 19, France. E-mail: deroux@rdebre.inserm.fr.

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