

A novel loss of function mutation in exon 10 of the FSH receptor gene causing hypergonadotrophic hypogonadism: clinical and molecular characteristics

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BACKGROUND: Inactivating mutations of the FSH receptor (FSHR) are a rare cause of hypergonadotrophic hypogonadism in women. Only one patient with primary amenorrhoea due to an *FSHR* gene mutation has been reported outside of Finland, where the prevalence of Ala189Val mutations is particularly high. **METHODS AND RESULTS:** Here, we describe the clinical, molecular genetic and functional characteristics associated with a novel inactivating mutation in exon 10 of the *FSHR* gene identified in a patient who presented with primary amenorrhoea at 17 years of age. The C to G transversion found at nucleotide 1043 causes a Pro348Arg substitution in the extracellular region of the FSHR and results in a mutant FSHR that is completely inactive in functional studies and that does not bind FSH. The proband exhibits apparent homozygosity for this recessive mutation. Her father is heterozygous for the mutation while analysis of exon 10 of the *FSHR* gene from her mother revealed only wild-type sequence. Chromosome painting was used to exclude deletions or rearrangements of 2p, and microsatellite markers did not show paternal uniparental isodisomy for this region. These findings suggest that the proband is hemizygous, with an inherited or de-novo microdeletion, or alternatively a de-novo gene conversion, of the accompanying *FSHR* allele. **CONCLUSIONS:** This case confirms the importance of the FSHR in female pubertal development and reproduction, and supports a relationship between phenotype and function for *FSHR* mutations.

Key words: FSH/FSH receptor/infertility/ovary/puberty

Introduction

The pituitary glycoprotein hormone FSH and its receptor (FSHR) are essential for normal reproductive function in both sexes (Simoni *et al.*, 1997; Themmen and Huhtaniemi, 2000). In the female, FSH regulates follicle maturation and stimulates estrogen production by granulosa cells in the ovary. In the male, FSH supports spermatogenesis by Sertoli cells (Simoni *et al.*, 1997; Tapanainen *et al.*, 1997; Themmen and Huhtaniemi, 2000). Targeted deletion of *Fsh* or the *Fshr* in female mice causes an arrest in follicular maturation at the preantral stage of development (Dierich *et al.*, 1998; Abel *et al.*, 2000). Patients with inactivating mutations in *FSHβ* or the *FSHR* have variable abnormalities of pubertal development with primary or secondary amenorrhoea (Aittomäki *et al.*, 1995; Beau *et al.*, 1998; Touraine *et al.*, 1999; Doherty *et al.*, 2002). However, the number of these naturally occurring mutations reported to date in humans is small, consistent with a recessive mode of transmission and a deleterious effect on fertility.

The G protein-coupled FSHR receptor consists of seven transmembrane domains and an amino-terminal extracellular hormone binding region (Simoni *et al.*, 1997; Themmen and Huhtaniemi, 2000). The gene encoding FSHR is located on the short arm of chromosome 2 in humans and consists of 10 exons spanning 54 kB of genomic DNA. The first 9 exons of this gene encode the extracellular domain of the FSHR. Exon 10 encodes the transmembrane and intracellular domains, as well as the proximal portion of the extracellular domain.

The first inactivating mutation of the *FSHR* gene was described in individuals from several Finnish relatives who presented with poorly developed secondary sexual characteristics, primary amenorrhoea and recessively inherited hypergonadotrophic ovarian failure (Aittomäki *et al.*, 1995, 1996). Functional studies demonstrated that this Ala189Val mutation results in a functionally inactive FSHR, consistent with the severe phenotype reported in affected patients (Aittomäki *et al.*, 1995). More recently, three patients with compound hetero-

zygous mutations in the FSHR have been described. Two patients (harbouring the mutations Asp224Val/Leu601Val and Ala189Val/Ala419Thr) had normal secondary sexual characteristics and primary amenorrhoea (Touraine *et al.*, 1999; Doherty *et al.*, 2002). The other patient (Ile160Thr/Arg573Cys) had a milder phenotype consisting of normal secondary sexual development and secondary amenorrhoea (Beau *et al.*, 1998). In this report, we describe a novel complete loss-of-function mutation in the FSHR in an adolescent girl with hypergonadotrophic ovarian failure and primary amenorrhoea.

Materials and methods

Mutational analysis of the FSHR gene

After written informed consent was obtained, genomic DNA was extracted from peripheral leukocytes using a nucleon BACC2 kit (Scott Lab Bioscience, Buckinghamshire, UK). The entire coding sequence of the *FSHR* gene was PCR-amplified using specific primer pairs. The exon 10 mutation was identified with the following primers: FSHF1, 5'-GCAATGAAGTGGTGGACGTGACCT-3', FSHR1, 5'-TG TAGCTGCTGATGCCAAAGATGG-3'. The 3' single nucleotide polymorphism was identified with: FSH3'F2, 5'-GAGTTAAGTGACTTGTTCA-3', FSH3'R2, 5'-CTGAGCCCTGCTTACATTC-3'. PCR reactions were performed using 200 ng of template DNA, 1 mmol/l MgCl₂, 100 ng of each primer, 100 µmol/l of each dNTP, 1.5 IU Taq Polymerase and 1× Super Taq Buffer (Kramel Biotech, Northumberland, UK) in a total volume of 50 µl. Reaction conditions were an initial denaturation step of 3 min at 93°C, followed by 30 each of 93, 58 and 72°C for 30 cycles, with a final extension of 5 min at 72°C. The PCR-amplified products were purified using a Wizard DNA Purification System (Promega, Madison, WI, USA) and were sequenced on an ABI377 Sequencer. Results were compared with the previously published *FSHR* gene sequence using sequence navigator software (Applied Biosystems, Foster City, CA, USA). The mutation was confirmed by digesting 15 µl of PCR product overnight at 37°C with 15 IU of *DdeI* (Promega). Digested products were analysed on an 8% acrylamide gel. DNA from 141 normal individuals was also PCR-amplified (primers FSHF1/FSHR1) and the products digested with this enzyme as a control.

Microsatellite analysis

Microsatellite analysis was performed using fluorescently labelled primers to amplify highly polymorphic regions around the *FSHR* locus (2p11–25.3). PCR products were electrophoresed using an ABI373 sequencer and results were analysed using genescan and genotyper software (Applied Biosystems).

Chromosome painting

Whole chromosome painting by fluorescence in-situ hybridization (FISH) was performed on fixed metaphase chromosome preparations from family members using chromosome 2 specific probes (Quest Diagnostics, Nichols Institute, San Juan Capistrano, CA, USA).

Construction of hFSHR expression vectors

Wild-type (WT) and mutant (Pro348Arg) FSHR expression vectors were created for use in functional studies. The wild-type vector was constructed by cloning hFSHR cDNA directly into a pSVL expression vector (Amersham Pharmacia Biotech, Piscataway, NJ, USA). The mutant vector was constructed by site-directed mutagenesis, using an overlapping PCR strategy with wild-type hFSHR cDNA as a template. This mutant cDNA fragment was ligated into the full-length hFSHR

and transferred into a pSVL expression vector. The presence of the mutation was confirmed by sequencing.

Functional studies

Cell culture experiments were performed using tsa201 cells, a human embryonic kidney line. These cells were grown in DMEM supplemented with 10% fetal bovine serum, 100 IU/ml penicillin and 100 µg/ml streptomycin in a 5% CO₂ atmosphere at 37°C.

Intracellular cAMP assays

Cells were transiently transfected with 500 ng of either negative control (empty vector), wild-type FSHR construct (WT) or mutant construct (Pro348Arg) by calcium phosphate DNA precipitation. After 48 h, cells were treated with 0.2 mmol/l 3-isobutyl-1-methyl-xanthine (IBMX) and varying concentrations of human FSH (0–400 IU/l, IRP-68/140; Sigma–Aldrich, St Louis, MO, USA) for 30 min. Media were removed, cells were snap-frozen on dry ice, and 750 µl cold 0.1 mol/l HCl was added to each well. Cell lysates were centrifuged (16 000 g) for 10 min at 4°C and 10 µl of each supernatant was removed and neutralized with an equal volume of 150 mmol/l Tris–HCl (pH 8.0). Cyclic AMP concentrations were measured by radioimmunoassay (Biomedical Technologies Inc., Stoughton, MA, USA) according to the manufacturer's instructions. Representative data for triplicate transfections (mean ± SEM) are shown.

Transient gene expression studies using a cAMP-responsive luciferase reporter

Cells were transiently transfected with 50 ng pSVL expression vector (empty vector, WT or Pro348Arg) and 20 ng pA3α846luc (cAMP-responsive) reporter. After 48 h, cells were treated with 0.2 mmol/l IBMX and varying concentrations of human FSH (0–400 IU/l) for 6 h. Cell extracts were prepared and luciferase assays performed. Representative data for triplicate transfections (mean ± SEM) are shown.

Hormone binding studies

Transfection

Monkey kidney COS-7 cells were transiently transfected with expression vectors containing either wild type or mutated *FSHR*, or the vector only using FuGENE 6 Transfection Reagent (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instructions. Co-transfections with a luciferase-expressing vector were done to control the transfection efficiency.

FSH binding assay

Recombinant human FSH (rhFSH; Organon, Oss, The Netherlands) was radioiodinated with Na^[125I]iodine (IMS 300; Amersham Biosciences UK Ltd, Little Chalfont, UK) using a lactoperoxidase method (Karonen *et al.*, 1975) to specific activity of 3800 cpm/ng. The transfected COS-7 cells were cultured in 9 cm diameter cell culture plates for 48 h, washed with phosphate-buffered saline and scraped into Dulbecco's PBS containing 0.1% bovine serum albumin (BSA; Sigma–Aldrich). Triplicate aliquots of cell suspensions, containing 2×10⁵ cells, were incubated in the presence of increasing amounts of radio-iodinated rhFSH (from 3.3 to 131.6 ng) in a total volume of 300 µl. For non-specific binding, 2.5 IU of rhFSH was used at each hormone concentration. Cells were incubated overnight at room temperature, and the radioactivity of the cell pellets was counted in a γ-spectrometer.

Results

Clinical details

The patient presented at 17 years with delayed puberty and primary amenorrhoea. She had first noted pubic hair development at 13 years of age and breast development at 14 years of age. She was otherwise healthy and had an unremarkable past medical history. Her mother and elder sister experienced menarche at 14 years and both had regular menstrual cycles (Figure 1a). Examination was unremarkable and pubertal assessment revealed Tanner stage 3, breast development, Tanner stage 4 pubic hair and normal female external genitalia. Her body mass index (19.6 kg/m²) was within normal limits (height, 160.6 cm, -0.6 SD; weight, 50.8 kg; midparental height, -1.1 SD), her bone age was delayed ~2.5 years and her lumbar spine bone mineral density was >2 SD below the age matched mean.

Endocrine investigations were consistent with a diagnosis of hypergonadotrophic hypogonadism (Table I). Her karyotype was normal (46,XX) and ovarian antibodies were negative. Diagnostic laparoscopy revealed normal but relatively immature Müllerian duct-derived structures (cervix, uterus and Fallopian tubes) and bilateral streak ovaries were reported. However, pelvic magnetic resonance imaging confirmed the presence of ovarian tissue containing follicles. Both parents had gonadotrophin levels within the normal range. Given the elevated gonadotrophins and the clinical phenotype, *FSHR* was considered a candidate gene in this patient.

***FSHR* gene sequence**

Sequencing revealed an apparently homozygous C to G transversion at nucleotide 1042 within exon 10 of the proband's *FSHR* gene (Figure 2). This change results in a Pro348Arg mutation and was not present in any of the 141 control subjects studied. The proband's father and sister are heterozygous carriers of the mutation. Her mother appears to have only wildtype exon 10 sequence. These genotypes were confirmed by restriction enzyme digestion (Figure 1b).

A similar inheritance pattern was detected for a single nucleotide polymorphism (SNP) in the genomic sequence that lies 3' to exon 10 of the FSH receptor. This SNP is located 1718 nucleotides from the exon 10 mutation within the 3' untranslated region. The proband's father is heterozygous at this site (C/T) whereas the proband and her mother appear to be homozygous/hemizygous for C and T respectively.

Microsatellite analysis

Microsatellite analysis showed that the proband is heterozygous for nine out of fourteen polymorphic markers spanning 2p (Table II). Segmental paternal uniparental isodisomy involving the D2S123 marker, a known region of chromosomal instability, was excluded as a cause of the presence of only C1043G mutant sequence in the proband. The microsatellite analysis also excludes the possibility that the proband inherited a large deletion within chromosome 2p from the mother.

Chromosome painting

Whole chromosome 2 painting by FISH failed to detect any chromosomal rearrangements in the proband or her parents (data not shown).

Table I. Endocrine evaluation of the proband

Hormone	Level	Reference range
FSH	105.1 IU/l	Post-menopausal >30 IU/l
LH	36.3 IU/l	
Estradiol	76 pmol/l	
Progesterone	3 nmol/l	>25 nmol/l indicates ovulation
Testosterone	1.6 nmol/l	0.3–2.5 nmol/l
Prolactin	160 mIU/l	0–450 mIU/l
IGF-I	24 nmol/l	9–48 nmol/l

IGF-I = insulin-like growth factor-I.

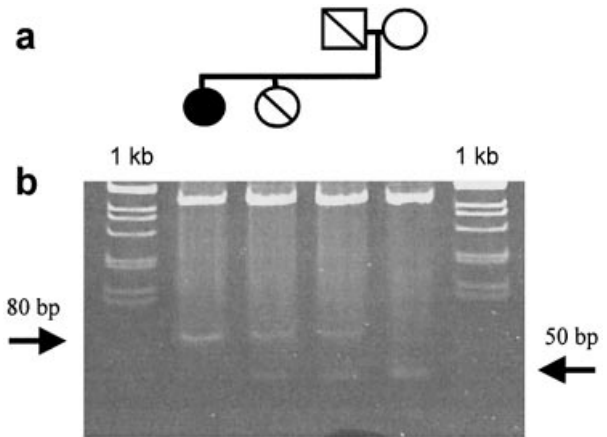


Figure 1. (a) Family pedigree. (b) Restriction enzyme analysis of a PCR-amplified fragment of the *FSHR* from the proband and other family members. The C1043G mutation changes a *DdeI* site, resulting in an 80 bp product following digestion with this enzyme in the presence of the mutation as opposed to a 50 bp product with wild-type sequence.

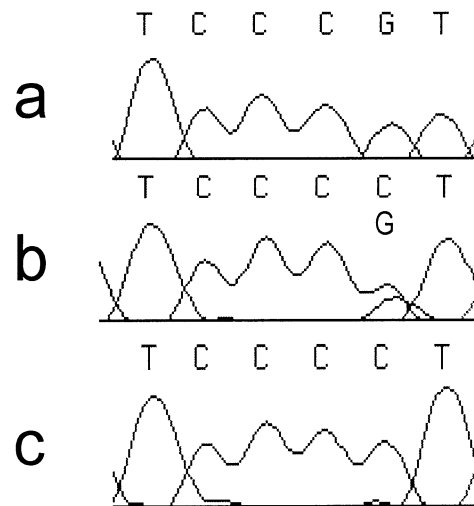


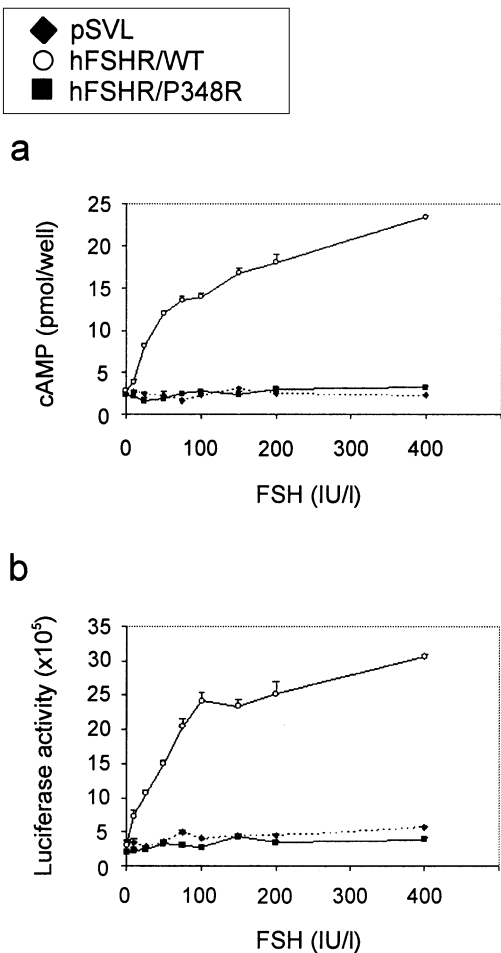
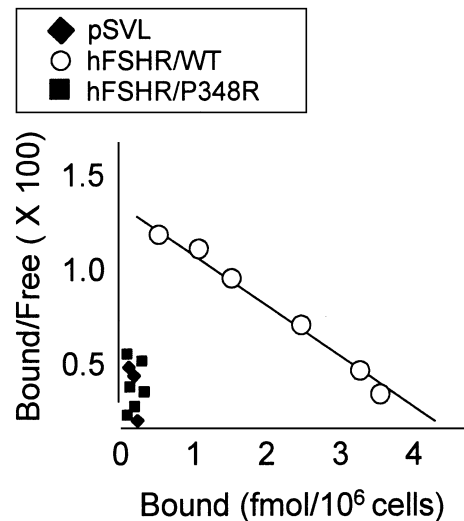
Figure 2. Chromatograms from DNA sequencing demonstrating a C1043G transition in exon 10 of the *FSHR* gene. (a) The proband appears homozygous for this mutation, (b) the father is heterozygous mutant/wild type, whereas (c) analysis of maternal exon 10 demonstrates only wild-type sequence.

Functional studies

Functional studies demonstrated a dose-dependent increase in luciferase activity and cAMP production with the WT *FSHR* in response to FSH stimulation (Figure 3). In contrast, the

Table II. Microsatellite analysis of polymorphic markers on chromosome 2p

Marker	Distance from pTel (kbp) ^a	Mother	Father	Proband
D2S281	5614	247, 249	245, 247	245, 247
D2S1358	16287	157, 160	157, 163	157
D2S175	16615	139, 141	133, 135	135, 139
D2S165	27779	151	143	143, 151
D2S177	37851	290	298, 302	290, 298
D2S119	45853	219, 223	223	223
D2S391	46436	144, 150	144, 150	144, 150
D2S288	46570	281, 283	281, 283	283
D2S2227	47441	207, 209	207	207, 209
FSHR	49568			
D2S1248	51138	337, 341	337, 349	337
D2S123	51826	210, 218	210, 226	218, 226
D2S378	58145	213, 217	213, 217	213, 217
D2S134	69837	315, 334	315, 326	326, 334
D2S139	80664	127	110, 127	127

^aBuild 27 of MapView, National Center for Biotechnology Information.**Figure 3.** Functional studies of the wild-type and mutant *FSHR*. (a) FSH-stimulated cAMP production. (b) FSH-stimulated activity of a cAMP responsive promoter linked to luciferase. Cells were transiently transfected with either vector alone (pSVL, diamonds), wild-type *FSHR* expression construct (hFSHR/WT, open circles) or mutant *FSHR* construct (hFSHR/P348P, closed squares). Data represent means \pm SEM of a typical experiment.**Figure 4.** Receptor binding studies. Scatchard analysis of [¹²⁵I]iodo-hFSH binding to either vector alone (pSVL, diamonds), wild-type *FSHR* expression construct (hFSHR/WT, open circles) or mutant *FSHR* construct (hFSHR/P348P, closed squares) transfected into COS-7 cells. Data represent means of triplicate experiments.

Pro348Arg mutant *FSHR* showed complete loss of activity at all FSH concentrations.

Receptor binding studies

In the binding assay, the occasional, non-linear low binding seen with the mutated *FSHR* could not be distinguished from that of the empty control vector. The equilibrium association constant (K_a) of FSH binding for the wild type receptor was $7.8 \pm 3.4 \times 10^9$ mol/l ($n = 3$ individual measurements), and the specific binding was 5.02 ± 0.12 fmol/10⁶ cells, consistent with previous studies (Figure 4).

Discussion

In this report, we describe a novel inactivating Pro348Arg mutation in the *FSHR* in a girl who presented with delayed pubertal development and primary amenorrhoea at 17 years of age. This mutation involves a hydrophobic proline residue in the extracellular domain of the *FSHR* that is highly conserved in the *FSHR* of other species, as well as in the human LH and thyroid-stimulating hormone receptors. Substitution of this proline with a hydrophilic arginine results in a mutant *FSHR* that has complete loss of function in in-vitro studies of receptor activity and is unable to bind hormone. These findings are consistent with previous studies showing that extracellular domain mutations result in impaired receptor expression and trafficking (Touraine *et al.*, 1999; Doherty *et al.*, 2002; Rannikko *et al.*, 2002).

Only a limited number of *FSHR* mutations have been reported to date (Figure 5). The best characterized of these is the Ala189Val (A189V) mutation that is found in the Finnish population (Aittomäki *et al.*; 1995, 1996; Tapanainen *et al.*, 1997; Rannikko *et al.*, 2002). Similar to our observations with the Pro348Arg (P348R) substitution, the Ala189Val mutation causes complete loss of receptor function, resulting in severe

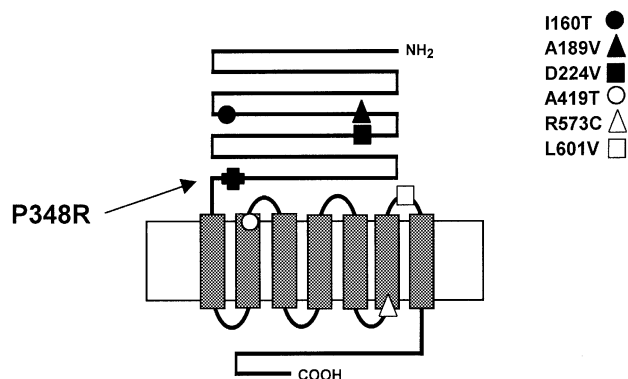


Figure 5. Schematic illustration of FSHR domain structure highlighting position of inactivating mutations described to date.

clinical features such as impaired pubertal development, primary amenorrhoea, low estradiol and elevated FSH in homozygous females. It is likely, therefore, that these cases represent the most extreme end of the phenotypic spectrum associated with FSHR mutations in women.

In contrast, compound heterozygous mutations in the FSHR have been found in three patients. Two of these patients developed normal secondary sexual characteristics but had primary amenorrhoea (Asp224Val/Leu601Val; Ala189Val/Ala419Thr) (Touraine *et al.*, 1999; Doherty *et al.*, 2002). The other patient has a milder phenotype consisting of normal secondary sexual development and secondary amenorrhoea (Ile160Thr/Arg573Cys) (Beau *et al.*, 1998). Although the Ala189Val, Asp224Val and Ile160Thr and Ala419Thr mutations were shown to cause complete loss of function in cAMP assays, the residual activity of the Leu601Val and Arg573Cys mutant receptors was 12 and 24% respectively (Beau *et al.*, 1998). Taken together with our data, these observations support a relationship between clinical phenotype and in-vitro receptor function, as has been proposed for the LH receptor, with 24% residual function in one mutant receptor associated with a delayed clinical presentation (Gromoll *et al.*, 1996). Furthermore, our data support the hypothesis that extracellular domain mutations are associated with marked loss of function and a severe phenotype, whereas transmembrane domain mutations can have milder effects (Doherty *et al.*, 2002). All heterozygous carriers of inactivating FSHR mutations reported to date have apparently normal pubertal development and fertility, suggesting that the presence of one wild-type FSHR allele is sufficient to permit normal reproductive function in humans.

A surprising feature of this pedigree is the absence of a mutation in the mother. The apparent homozygous genotype in the proband would suggest the inheritance of the same mutant allele (C1043G) from the mother and the father. Although the father was heterozygous for this change, the mother appeared to have wild-type sequence. A similar inheritance pattern was found for an SNP in a 3' region of genomic DNA located 1718 nucleotides from the exon 10 mutation. This inheritance pattern could be explained by one of three genetic mechanisms: paternal uniparental isodisomy (UPD), gene conversion, or deletion of part of the maternal allele.

Several cases of UPD involving chromosome 2 have been described, but these are usually whole chromosome changes that involve maternally inherited material in all cases (Kotzot, 1999). The presence of heterozygosity for several markers in the proband would exclude this. More recently, a case of maternal segmental UPD has been described that involves a region of $\geq 100\,000$ base pairs of chromosome 2p and includes the marker D2S123 (Stratakis *et al.*, 2001). This marker lies in the vicinity of the FSHR locus [GeneMap '99 (GB4), National Center for Biotechnology Information] and represents a region of high chromosomal recombination and potential instability. However, microsatellite analysis of our pedigree using this marker (D2S123) excludes paternal segmental UPD in this region as the cause of the genotype in the proband. It remains possible that a more limited region of paternal segmental UPD or somatic gene conversion has occurred (involving the FSHR exon 10 locus and region 3' to it), in which the mutant allele (from the father) undergoes genetic exchange with the wild-type allele (from the mother). The phenomenon of gene conversion is becoming increasingly recognized in humans (Jonkman *et al.*, 1997; Merke *et al.*, 1999).

The presence of heterozygous markers in the proband at positions D2S123 (cen) and D2S2227 (tel) exclude the presence of a very large deletion of a maternal allele involving the FSHR locus. Similarly, chromosome painting failed to detect any significant deletions or rearrangements. It remains possible that the proband harbours a small deletion (spontaneous or inherited from the mother) of this region of chromosome 2 that was not detected by our analysis. Indeed, a combined mutation/deletion has been reported in a patient with an inactivating LH receptor mutation previously (Laue *et al.*, 1996). Given the inheritance pattern of the SNP in the 3' region, such a deletion would have to extend beyond the 3' boundary of exon 10 of the FSH receptor locus.

Although FSHR mutations appear to be rare in most populations (Layman *et al.*, 1998; Jiang *et al.*, 1998; Conway *et al.*, 1999), this report confirms that FSHR mutations can be found in patients who present with spontaneous (non-familial) delayed puberty and primary amenorrhoea. The clinical and molecular features of these patients further our understanding of genotype-phenotype correlations in this disorder and the role of the FSHR in the development and function of the ovary.

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References

- Abel, M.H., Wootton, A.N., Wilkins, V., Huhtaniemi, I., Knight, P.G. and Charlton, H.M. (2000) The effect of a null mutation in the follicle-stimulating hormone receptor gene on mouse reproduction. *Endocrinology*, **141**, 1795–1803.
- Aittomäki, K., Dieguez Lucena, J.L., Pakarinen, P., Sistonen, P., Tapanainen, J., Gromoll, J., Kaskikari, R., Sankila, E.M., Leivaslaiho, H., Engel, A.R.

- et al.* (1995) Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell*, **82**, 959–968.
- Aittomäki, K., Herva, R., Stenman, U.-H., Juntunen, K., Ylostalo, P., Hovatta, O. and de la Chapelle, A. (1996) Clinical features of primary ovarian failure caused by a point mutation in the follicle-stimulating hormone receptor gene. *J. Clin. Endocrinol. Metab.*, **81**, 3722–3726.
- Beau, I., Touraine, P., Meduri, G., Gougeon, A., Desroches, A., Matuchansky, C., Milgrom, E., Kuttann, F. and Misrahi, M. (1998) A novel phenotype related to partial loss of function mutations of the follicle stimulating hormone receptor. *J. Clin. Invest.*, **102**, 1352–1359.
- Conway, G., Conway, E., Walker, C., Hoppner, W., Gromoll, J. and Simoni, M. (1999) Mutation screening and isoform prevalence of the follicle-stimulating hormone receptor gene in women with premature ovarian failure, resistant ovary syndrome and polycystic ovary syndrome. *Clin. Endocrinol.*, **51**, 97–99.
- Dierich, A., Ram Sairam, M., Monaco, L., Fimia, G.M., Gansmuller, A., LeMeur, M. and Sassone-Corsi, P. (1998) Impairing follicle-stimulating hormone (FSH) signaling *in vivo*: targeted disruption of the FSH receptor leads to aberrant gametogenesis and hormonal imbalance. *Proc. Natl Acad. Sci. USA*, **95**, 13612–13617.
- Doherty, E., Pakarinen, P., Tiitinen, A., Kiilavuori, A., Huhtaniemi, I., Forrest, S. and Aittomäki, K. (2002) A novel mutation in the FSH receptor inhibiting signal transduction and causing primary ovarian failure. *J. Clin. Endocrinol. Metab.*, **87**, 1151–1155.
- Gromoll, J., Simoni, M., Nordhoff, V., Behre, H.M., De Geyter, C. and Nieschlag, E. (1996) Functional and clinical consequences of mutations in the FSH receptor. *Mol. Cell. Endocrinol.*, **125**, 177–182.
- Jiang, M., Aittomäki, K., Nilsson, C., Pakarinen, P., Iitii, A., Torresani, T., Simonsen, H., Goh, V., Pettersson, K., de la Chapelle, A. and Huhtaniemi, I. (1998) The frequency of an inactivating point mutation (⁵⁶⁶C→T) of the human follicle-stimulating hormone receptor gene in four populations using allele-specific hybridization and time-resolved fluorometry. *J. Clin. Endocrinol. Metab.*, **83**, 4338–4343.
- Jonkman, M.F., Scheffer, H., Stulp, R., Pas, H.H., Nijenhuis, M., Heeres, K., Owaribe, K., Pulkkinen, L. and Uitto, J. (1997) Revertant mosaicism in epidermolysis bullosa caused by mitotic gene conversion. *Cell*, **88**, 543–551.
- Karonen, S.-L., Mörsky, P., Sirén, M. and Seuderling, U. (1975) An enzymatic solid-phase method for trace iodination of proteins and peptides with ¹²⁵I-iodine. *Anal. Biochem.*, **67**, 1–10.
- Kotzot, D. (1999) Abnormal phenotypes in uniparental disomy (UPD): fundamental aspects and a critical review with bibliography of UPD other than 15. *Am. J. Med. Genet.*, **82**, 265–274.
- Laue, L.L., Wu, S.-M., Kudo, M., Bourdony, C.J., Cutler, G.B., Hsueh, A.J. and Chan, W.Y. (1996) Compound heterozygous mutations of the luteinizing hormone receptor gene in leydig cell hypoplasia. *Mol. Endocrinol.*, **10**, 987–997.
- Layman, L.C., Made, S., Cohen, D.P., Jin, M. and Xie, J. (1998) The Finnish follicle-stimulating hormone receptor gene mutation is rare in North American women with 46,XX ovarian failure. *Fertil. Steril.*, **69**, 300–302.
- Merke, D.P., Tajima, T., Baron, J. and Cutler, G.B. Jr (1999) Hypogonadotropic hypogonadism in a female caused by an X-linked recessive mutation in the *DAX1* gene. *N. Engl. J. Med.*, **340**, 1248–1252.
- Rannikko, A., Pakarinen, P., Manna, P., Beau, I., Misrahi, M., Aittomäki, K. and Huhtaniemi, I. (2002) Functional characterization of the human FSH receptor with an inactivating Ala189Val mutation. *Mol. Hum. Reprod.*, **8**, 311–317.
- Simoni, M., Gromoll, J. and Nieschlag, E. (1997) The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr. Rev.*, **18**, 739–773.
- Stratakis, C., Taymans, S.E., Schteingart, D. and Haddad, B.R. (2001) Segmental uniparental isodisomy (UPD) for 2p16 without clinical symptoms: implications for UPD and other genetic studies of chromosome 2. *J. Med. Genet.*, **38**, 106–109.
- Tapanainen, J.S., Aittomäki, K., Min, J., Vaskivo, T. and Huhtaniemi, I.T. (1997) Men homozygous for an inactivating mutation of the follicle-stimulating hormone (FSH) receptor gene present variable suppression of spermatogenesis and fertility. *Nature Genet.*, **15**, 205–206.
- Themmen, A.P.N. and Huhtaniemi, I.T. (2000) Mutations of gonadotropins and gonadotropin receptors: elucidating the physiology and pathophysiology of pituitary–gonadal function. *Endocr. Rev.*, **21**, 551–583.
- Toledo, S.P. (1999) Inactivating mutations of the LH receptor gene: more than two different phenotypes. *Eur. J. Endocrinol.*, **140**, 186.
- Touraine, P., Beau, I., Gougeon, A., Meduri, G., Desroches, A., Pichard, C., Detoef, M., Paniel, B., Prieur, M., Zorn, J.R. *et al.* (1999) New natural inactivating mutations of the follicle-stimulating hormone receptor: correlations between receptor function and phenotype. *Mol. Endocrinol.*, **13**, 1844–1854.

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