Toward gene therapy of primary ovarian failure: adenovirus expressing human FSH receptor corrects the Finnish C566T mutation

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Resistance ovarian syndrome is a heterogeneous disorder inherited as a Mendelian recessive trait and characterized by infertility, primary amenorrhea, normal karyotype and elevated serum FSH and LH levels. An inactivating mutation, C566T, in FSH receptor gene (FSHR) has been identified initially in Finland. We investigated if an adenovirus expressing a normal copy of human FSHR (Ad-hFSHR) has the ability to: (i) transfected granulosa cell lines, (ii) render the transfected cell lines responsive to FSH stimulation and (iii) transcomplement the malfunctioning form of human FSHR gene with C566T mutation. COS-7, JC-410, JC-410-P450-sec-luc and JC-410-StAR-luc cell lines were infected by Ad-hFSHR followed by treatment with FSH. Functional activity of the Ad-hFSHR was tested by measuring cyclic adenosine monophosphate (cAMP) or luciferase activity in response to FSH stimulation, and showed 2–4.6-fold increases in Ad-hFSHR transfected cells compared with untransfected or Ad-LacZ transfected cells, indicating that Ad-hFSHR is functionally active and expressing hFSHR. Generation of cAMP in cells expressing only mutated hFSHR-T566 showed minimal increase after FSH stimulation. Co-transfection of Ad-hFSHR in these cells carrying the malfunction form of human FSHR caused significant increases of 2.2–7.4-fold in FSH dependent cAMP generation (P = 0.0007). We concluded that adenovirus expressing a normal human FSHR can compensate the inactivating human FSHR-C566T mutation and restore FSH responsiveness.

Keywords: Finnish C566T mutation; follicle-stimulating hormone receptor; gene therapy of ovarian failure; infertility; primary amenorrhea

Introduction

Genetic impairments of the hypothalamo–pituitary–gonadal axis can cause infertility and hypogonadism (Beau et al., 1992). Kallmann’s syndrome is an example of this type of hypothalamic defect (Bick et al., 1992; Hardelin et al., 1992). Mutations in the FSH and LH genes lead to a pituitary level defect (Weiss et al., 1992; Matthews et al., 1993; Layman et al., 1997). FSH and LH are members of the glycoprotein hormone family, which also includes human chorionic gonadotropin (hCG) and thyrotropic hormone (TSH). These are heterodimeric hormones, which in a given species, consisting of a common α subunit and a β subunit, which drives functional specificity of each hormone (Pierce and Parsons, 1981; Bousfield et al., 1994). Both hCG and human FSH hormones have crystal structures that reveal elongated molecules with similar folds for the α and β subunits (Lapthorn et al., 1994; Wu et al., 1994; Fox et al., 2001; Fan and Herdrickson, 2005). In the case of FSH and its receptor (Fan and Herdrickson, 2005) showed that the hormone is bound in a hand-clasp fashion to an elongated, curved receptor. These glycoprotein hormones act through their specific G-protein-coupled receptors on the target cell surfaces (Simoni et al., 1997). Protein structures of the receptors are characterized by a large extracellular domain, involved in the specificity and high affinity of hormone binding, a seven transmembrane domain, responsible for receptor activation and signal transduction, and a short carboxyl terminal intracellular domain (Simoni et al., 1997). The distinctive feature of FSH receptor (FSHR), LH receptor (LHR) and TSH receptor (TSHR) is their large extracellular ligand-binding domain (Aittomaki et al., 1995). This large extracellular N-terminal portion of the receptors is composed of two cystine clusters flanking nine lucine-rich repeats which form a succession of β-strand and α-helices organized into horseshoe-shaped structure (Smits et al., 2003). It has been proposed that glycoprotein hormones interact with residues of the α-strands making the concave surface of the horseshoe (Smits et al., 2003).

FSH and LH are essential for gonadal maturation and development and for gamete production during the prepubertal and fertile phases of life in both males and females (Simoni et al., 1997). These hormones...
are synthesized in the same cells of the pituitary (the gonadotrophs) (Pierce and Parsons, 1981; Bousfield et al., 1994), and act through their corresponding receptors: the FSHR and LHR. FSHRs are localized on the surface of Sertoli cells in the testes and granulosa cells in the ovary (Pierce and Parsons, 1981; Bousfield et al., 1994; Simoni et al., 1997). Its response to FSH is considered to be essential for follicular development and maturation in female, and for initiation of spermatogenesis and maintenance of quantitatively normal sperm production in adult males (Tapanainen et al., 1997). Folliculogenesis and ovulation are arrested in the prepubertal ages, and they are induced by FSH stimulation through FSHR at puberty. Lack of FSH secretion or a defective FSHR can arrest ovarian maturation at puberty. The difference between the two conditions (FSHR defect or FSH secretion defect) is the elevated FSH in the absence of FSHR response, due to lack of gonadal negative feedback. This condition also named hypergonadotropic hypogonadism (Aittomaki et al., 1995). Primary amenorrhea, variable degrees of development of secondary sex characterizations, normal karyotype and normal prepubertal developments are common clinical findings in this condition. Aittomaki et al. (1995) showed that an inactivating mutation in exon 7 of the FSHR gene causes ovarian failure due to resistant ovary syndrome (ROS). The disorder is heterogeneous with autosomal recessive mode of inheritance in most cases (Simpson, et al., 1971). Affected females are infertile, and there is no treatment at the present time, other than symptomatic relief with hormone replacement therapy. This condition is devastating for women who are interested in becoming pregnant. There is little chance of these women achieving a spontaneous pregnancy. Ovulation stimulation with human menopausal gonadotropin treatment has been unsuccessful. The only method for women with ROS to become pregnant is through using donated eggs. Egg donation, however, is prohibitively expensive, and many women are ethically opposed to this method. Additionally, the child will not be genetically related to at least one of the parents.

FSHR in humans consists of 10 exons spanning 85 kb of genomic DNA (Sprengel et al., 1990; Minegishi et al., 1991; Kelton et al., 1992), located on chromosome 2p21–p16 (Rousseau-Mercier et al., 1993; Gromoll et al., 1994). Exons 1–9 encode the extracellular domain of the protein and exon 10 encodes the transmembrane domain, the intracellular domain and the proximal portion of the extracellular domain (Simoni et al., 1997). The first homozygous mutation identified in the FSHR gene was an inactivating point mutation (C566T) in exon 7 resulting in an ala189val change (Aittomaki et al., 1995). This missense mutation occurs in a region encoding the extracellular ligand-band binding domain of the receptor and located in a highly conserved region of FSHR in humans and other species such as monkey, sheep and rat (Sprengel et al., 1990; Gromoll et al., 1993; Yarney et al., 1993), which suggests that it must be functionally important. Transfection experiments showed a dramatic decrease in the FSHR response to FSH by reducing cyclic adenosine monophosphate (cAMP) production and binding capacity (Aittomaki et al., 1995). To date, variable abnormalities of pubertal development with primary or secondary amenorrhea in patients with a partial or total loss of functional mutations in different regions of the FSHR gene have been reported (Beau et al., 1998; Touraine et al., 1999; Doherty et al., 2002; Allen et al., 2003). Males homozygous for the mutation have variable degrees of spermatogenic failure but do not show azoospermia or absolute infertility (Tapanainen et al., 1997). In females a heterozygous state of mutated FSHR does not seem to affect ovarian function. In homozygous mutant women, follicular growth is arrested, follicle atresia continues and with the lack of normal cycling ovarian function, this leads to the syndrome of hypergonadotropic primary amenorrhea, infertility with normal internal and external genitalia (Aittomaki et al., 1995). This variation of the effect of the C566T mutation between men and women demonstrates that FSH has a more important function in females than in males (Tapanainen et al., 1997). In this study, we used a plasmid containing the point mutation (C566T) in human FSHR (pFSHR-T566) gene at exon 7. We showed that this inactive receptor has no response to FSH in various cell types. We have constructed an adenovirus vector expressing the normal human FSHR gene (Ad-hFSHR) and showed that various cell lines without any internal FSHR, when transfected with Ad-hFSHR, positively responded to FSH stimulation. Additionally, Ad-hFSHR restored FSH responsiveness to cells transfected with inactivating pFSHR-T566, indicating that Ad-hFSHR responds to FSH and can functionally transcomplement C566T-mutated hFSHR.

Materials and methods

Cell culture

We used the COS-7, JC-410, JC-410-P450-scc-luc and JC-410-StAR-luc cell lines for this study. COS-7 cells are monkey kidney cells that are commonly used for FSHR expression studies (Gromoll et al., 1996; Simoni et al., 1999). The JC-410 cell line is a steriodogenetically stable porcine granulosa cell line that we have isolated and characterized extensively (Chedrese et al., 1998). JC-410-StAR-luc and JC-410-P450-scc-luc are genetically modified cell lines with gene constructs under the control of the StAR promoter or P450-side chain cleavage enzyme (SCC) promoter linked to a luciferase reporter gene as we have described previously (Gillio-Meina et al., 2000). These two promoters were selected because their activity is normally upregulated with FSH in the presence of normal FSHR (Gillio-Meina et al., 2000). All of these cell lines lack any endogenous FSHR activities (Simoni et al., 1997; Chedrese et al., 1998; Havelock et al., 2004).

The COS-7 cells were maintained in Dulbecco’s Modified Eagle Medium containing 4.5 g/l D-glucose and 2.0 mmol/l-l-glutamine (Invitrogen, Carlsbad, Calif) supplemented with 10% fetal bovine serum, 1.5 g/l sodium bicarbonate and 1% penicillin/streptomycin at 37 C and 5% CO2. The JC-410 cells were maintained in Medium 199 (Invitrogen) containing 2.0 mmol/l-l-glutamine, Earle’s salts, 2.2 g/l sodium bicarbonate supplemented with 10% newborn calf serum, 1% penicillin at 37 C and 5% CO2. Cells were cultured in standard microplates (tissue-culture grade) with cell concentrations of 105–106 cells/ml.

Assessment of adenovirus transfection efficiency

The ability of adenovirus to infect COS-7 and granulosa cells was determined by using an adenoviral vector that carries a marker gene, coding for bacterial β-galactosidase gene, Ad-LacZ (a kind gift from Dr Savio Woo, Mount Sinai School of Medicine, New York, NY), followed by X-gal staining using an in situ β-galactosidase staining kit (Stratagene, La Jolla, Calif) as we have reported previously (Al-Hendy et al., 2005).

Plasmids

A DNA fragment containing the human FSHR cDNA with encoding of the point mutation (C566T) in exon 7 of the hFSHR gene (a kind gift from Dr Huhtaniemi, Turku, Finland) was inserted in the mammalian expression vector pSG5 (Stratagene) to obtain inactivated hFSHR plasmid (pFSHR-T566). COS-7 and JC-410 cells were grown to 60–70% confluency in 100-mm plates and transfected with pFSHR-T566 using FuGENE 6 transfection reagents (Roche Molecular Biochemicals, Mannheim, Germany), according to manufacturer’s instructions. A plasmid carrying the gentamycin-resistant gene was cotransfected with pFSHR-T566. Twenty-four hours later, cells were transferred to 150-mm tissue culture plates and were cultured for 3 weeks in the presence of different concentrations (100, 300 and 500 ng/ml) of genetin (G-418). When visible colonies of cells (>1000 cells) were formed in the plates that contained pFSHR-T566 and no colonies were observed in the control plates, cells were picked up from the plates using sterile glass cloning cylinders (Southern and Berg, 1982) transferred to 6-well plates, and maintained with culture media containing the appropriate concentration of G418. Once cells reached 80–90% confluency, they
were split 1–3 and were used for further experiments. The presence or absence of hFSHR-T566 in selected colonies was reconfirmed by PCR.

**PCR for normal and mutant hFSHR**

DNA was extracted from the cultured cells using the DNeasy tissue kit (Qiagen, Valencia, Calif), according to the manufacturer’s protocol. Specific primer sets were designed for normal and mutant hFSHR. Based on CS66T mutation on exon 7, the 3-end of the direct primer was designed to be C, for normal, or T, for the mutant hFSHR. The forward primer, GGATTCAAGAAA-TACACAAATCCTGTG, was used for normal hFSHR amplification and GGATT-CAAGAAATACACAAATCCTGTG was used for the mutated hFSHR. The reverse primers, AGTTTGGCAGTCAATGGCCATAG and ACCATT-CATTGCTAAGTCAATGGCCATAG, were used to amplify normal and mutant hFSHR, respectively. RED Taq Ready-Mix PCR reaction containing 0.4 mM deoxynucleoside triphosphates, 20 mM Tris–HCl (pH 8.3) with 100 mM KCl, 3 mM MgCl2, 0.002% gelatin, stabilizers and 0.06 IU/μl Taq polymerase (Sigma Aldrich, St Louis, MO) was used for PCR amplification in a total volume of 50 μl of reaction containing 100 ng template DNA and 150 ng primers. PCR condition was set as a denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 45 s, 60–64 °C for 45 s and 72 °C for 1 min, followed by a final elongation step at 72 °C for 10 min. PCR products were analyzed in 1% agarose gel.

**Construction of adenovirus vector carrying normal hFSHR gene**

To construct the adenovirus expressing the normal hFSHR gene, we obtained hFSHR cDNA subcloned into the Pmel site of a pShuttle vector (kindly provided by Dr Cheryl Nechaman, David Axelrod Institute for Public Health, Wadsworth Center, Albany, NY). The pShuttle-CMV is a kanamycin-resistant plasmid under the control of the cytomegalovirus promoter (Stagrnage). The pShuttle-CMV-hFSHR was digested by the Pmel (Qiogene Inc., Montreal, Quebec, Canada) and purified with phenol/chloroform. The purified pShuttle-CMV-hFSHR vector was cotransfected with viral DNA [which contains the backbone of the adenovirus serotype 5 (Ad5)] into E. coli BJ5183 bacteria (Qiogene Inc.), where homologous recombination took place. The recombinants were selected by kanamycin. The construct was linearized with a PacI restriction enzyme and purified with phenol/chloroform extraction. The DNA was then used to transfect the adenovirus-permissive QBI-HEK-293 cells (Qiogene Inc.), which contain the rest of the Ad5 genome, using the calcium phosphate method as previously described (Bett et al., 1993). After 24 h, cells were washed, split and incubated at 37 °C with a 5% CO2 concentration. The following day, cells were overlaid with Seaplaque agarose and incubated for 2 weeks to pick viral plaques. Viral plaques were used to amplify normal and mutant hFSHR, respectively. RED Taq Ready-Mix PCR reaction containing 0.4 mM deoxynucleoside triphosphates, 20 mM Tris–HCl (pH 8.3) with 100 mM KCl, 3 mM MgCl2, 0.002% gelatin, stabilizers and 0.06 IU/μl Taq polymerase (Sigma Aldrich, St Louis, MO) was used for PCR amplification in a total volume of 50 μl of reaction containing 100 ng template DNA and 150 ng primers. PCR condition was set as a denaturation at 94 °C for 5 min, followed by 35 cycles at 94 °C for 45 s, 60–64 °C for 45 s and 72 °C for 1 min, followed by a final elongation step at 72 °C for 10 min. PCR products were analyzed in 1% agarose gel.

**Ad-hFSHR functional tests**

Ad-hFSHR function was tested by its ability to support an increase in cAMP concentration or luciferase activity in appropriate cell types. Precultured COS-7 and JC-410 cell lines at 70–80% confluency were infected by 100 PFU/cell of Ad-hFSHR or Ad-LacZ, as we have reported previously (Somers et al., 1999; Al-Hendy et al., 2005). Previous data also indicate effective transfection of murine (KK-15) granulosa cells with Ad-LacZ both in vitro and in vivo with 100% transfection of cells at 50–100 PFU/cell (Al-Hendy et al., 2005).

**Results**

**Adenovirus optimally transfects COS-7 and granulosa cells**

To assess the ability of adenovirus to transfect COS-7 and JC-410 porcine granulosa cell lines, we transfected the cells with Ad-LacZ, followed by X-gal staining and looked for β-galactosidase activity. These cells exhibited a bright nuclear blue color (Fig. 1), which suggests successful adenoviral transfection and β-galactosidase expression. Adenovirus has been shown to transfect the human granulosa cell line, the target cell for hFSHR expression (Somers et al., 1999; Al-Hendy et al., 2005). Previous data also indicate effective transfection of murine (KK-15) granulosa cells with Ad-LacZ both in vitro and in vivo with 100% transfection of cells at 50–100 PFU/cell (Al-Hendy et al., 2005).

**Identification of normal and mutated hFSHR genes in transfected cells**

We used PCR amplification with primers, either specific for the normal hFSHR gene or specific for an hFSHR gene copy that carries the inactivating Finnish CS66T mutation, to study the distribution of these genes in our target cells. None of these primer sets were able to show any amplicons with COS-7 or JC-410 cell lines (Fig. 2a and b).

When these cells were transfected with phFSHR-T566, they showed the expected bands when PCR was performed with the specific primer set for mutated hFSHR (Fig. 2a). The cells that were transfected with phFSHR-T566, followed by transfection with Ad-hFSHR and luciferase transactivation values reflected Ad-FSHR expression in the transfected cells. The results were normalized against the number of cells per well.

In order to test the transcomplement effect of the Ad-FSHR, COS-7 and JC-410 cells were transfected with mutated plasmid (phFSHR-T566) and were used in two different experiments. First, their cAMP production in response to human FSH was measured, as mentioned previously. In another experiment, transfected cells with mutated plasmid were cotransfected with 100 PFU/cell of Ad-hFSHR or Ad-LacZ, and then tested for the transcomplement effect of the Ad-hFSHR by measuring their cAMP production after stimulation with FSH.
Ad-hFSHR restores FSH responsiveness in COS-7 and JC-410 granulosa cells

The FSH-dependent cAMP production test indicated that untransfected COS-7 and JC-410 cell lines had no increase in cAMP generation after FSH stimulation, confirming prior reports that these two cell lines lack any endogenous FSHR activities (Simoni et al., 1997; Chedrese et al., 1998; Havelock et al., 2004) (Fig. 3). Transfection of these two cell lines with Ad-hFSHR induced a significant response to FSH signaling in COS-7 and JC-410 cell lines that exhibited a 3.7–4.6-fold increase in cAMP production, respectively, at a FSH concentration of 150 ng/ml (P = 0.002 for COS-7; P = 0.001 for JC-410 transfected with Ad-hFSHR) (Fig. 3). This marked increase in cAMP upon FSH stimulation clearly indicates that Ad-hFSHR expresses a functionally active hFSHR gene that revived FSH responsiveness in these cells that otherwise possess no endogenous FSHR activity. Transfection with Ad-LacZ did not show any significant increase in cAMP production in response to FSH, which indicates that the adenovirus itself had no effect on the cell response to FSH.

Luciferase activity

We proceeded to confirm the function of the adenovirus-delivered hFSHR in two additional specialized cell lines. We have previously described the construction of JC-410 porcine granulosa cell lines that express the luciferase marker gene under control of either the StAR promoter (JC-410-StAR-luc) or the P450-SCC gene promoter (JC-410-P450-ssc-Luc) (Chedrese et al., 1998; Gillio-Meina et al., 2000; Smida et al., 2004). The direct upregulation by FSH stimulation of these two promoters is well documented (Chedrese et al., 1998; Gillio-Meina et al., 2000; Havelock et al., 2004; Smida et al., 2004). We studied the effect of Ad-hFSHR transfection on the activity of these two promoters in porcine granulosa cells. The two cell lines showed minimal change in luciferase activity in response to different doses of FSH stimulation (Fig. 4). After transfection with Ad-LacZ, there was no significant increase in luciferase activity, confirming that the adenovirus itself had no effect on the cell response to FSH. Transfection with Ad-hFSHR, followed by FSH stimulation, revealed a 2-fold increase in luciferase activity in JC-410-StAR-scc-luc cell lines (P = 0.012) (Fig. 4) and a 3-fold increase in luciferase transactivation in JC-410-P450-ssc-luc cell lines (P = 0.001) (Fig. 4) compared with untransfected cell control.

Ad-hFSHR corrects the C566T inactivating mutation

As shown in Fig. 5, enzyme immunoassay indicated that the COS-7 and JC-410 cell lines expressing mutated hFSHR (hFSHR-T566) had no significant increase in cAMP production when treated with FSH, which indicates that the mutated hFSHR is functionally impaired. This confirmed previous reports suggesting that the C566T mutation inactivates FSH binding to hFSHR (Aittomaki et al., 1995). Similar results were observed in both the COS-7 and JC-410 cell lines that were cotransfected with Ad-LacZ and mutated plasmid (Fig. 5). This demonstrates that the presence of the adenovirus vector in these cells does not support any changes in the cell response to FSH. Conversely, when these cells were cotransfected with the malfunctioning receptor (phFSHR-T566) and Ad-hFSHR, followed by...
FSH treatment, they demonstrated a marked increase of 2.2–7.4-fold in cAMP production (Fig. 5). The significant increases \( (P = 0.006, P = 0.001) \) in the cAMP generation in those cells suggested successful compensation of the inactivating C566T mutation by the intact adenovirus-delivered hFSHR gene.

**Discussion**

Hypergonadotropic ovarian failure is a common cause of female infertility and accounts for about half of all cases with primary amenorrhea (Reindollar and McDonough, 1984; Timmreck and Reindollar, 2003). This disorder is a heterogeneous condition and describes women with amenorrhea, elevated circulating endogenous FSH and LH levels, normal karyotype, intact uterus and vagina, characteristic presence of numerous primordial follicles on ovarian biopsy and infertility (Simpson et al., 1971; Simpson and Plunkett, 1979; Aittomaki et al., 1995). The first homozygous mutation associated with this condition was reported by Aittomaki et al. (1995). It is an inactivating C566T mutation in exon 7 of FSHR gene located on chromosome 2p21–p16 predicting an Ala189Val change in the extracellular domain of the protein, which results in total function loss of the receptor. This mutation severely affects FSH-FSHR interaction, which is essential for normal reproductive development, particularly in females (Simpson et al., 1971). Defective FSHR response to FSH stimulation arrests ovarian follicles at the primordial stage in females homozygous for the C566T mutation (Aittomaki et al., 1995). Currently, there is no effective treatment for a woman suffering from this mutation that would enable them to achieve a normal pregnancy (Wentz, 1996).

In this study, we constructed an adenovirus expressing normal hFSHR. We showed that adenoviral vectors have the ability to transfect COS-7 and porcine JC-410 granulosa cell lines by using an adenoviral vector carrying the marker gene, Ad-LacZ. As the first step for ovarian failure gene therapy, COS-7, JC-410, JC-410-StAR-scc-luc and JC-410-P450-scc-luc cell lines were transfected by Ad-hFSHR followed by stimulation with different concentrations of FSH. FSH-dependent cAMP production and luciferase activity measurement indicated that our vector construct was functionally active to express the hFSHR gene in all transfected cells with 3.7–4.6-fold increases in the cAMP generation and 2–3-fold increases of luciferase activity in appropriate cells. More importantly, our data demonstrated that the Ad-hFSHR vector is able to correct the Finnish C566T mutation in those cells that express malfunctioning hFSHR with 2.2–7.4-fold increases in the cAMP generation. Our group, as well as others, attempted to perform hFSHR protein expression studies by western blot analysis but have consistently run into problems with antibody specificity. We have tried several commercial FSHR Abs, and also antibodies made by our collaborators, but several non-specific bands and backgrounds consistently appeared. This seems to be a common problem in the field.

A functional study of this inactivating mutation revealed a dramatic decrease in cAMP production, the first measurable response of FSHR...
to FSH stimulation (Aittomaki et al., 1995). In another study using the same plasmid with inactivating C566T mutation (Rannikko et al. 2002) ruled out any defective transcription or decreased mRNA stability of the mutated gene as a cause of the decreased receptor level by doing northern hybridization. They also concluded that the translation and/or degradation are not significantly affected by the mutation (Rannikko et al. 2002). In this study, we have used stably transfected cell lines using the same mutated plasmid (a kind gift from Dr Huhtaniemi, Turku, Finland) that has been shown to be functionally active in two different studies. Based on that, we are proposing that the increase of cAMP levels in response to FSH stimulation in the cells transfected with C566T mutated plasmid after transfection with Ad-FSHR (and not before that) suggest the successful expression of the normal hFSHR in the presence of C566T mutated FSHR. In other words, there is no dominant negative effect exerted by this mutation on the expression of normal FSHR. This is particularly important since some mutations work as an active dominant-negative variant that precludes correction even when an intact copy of the affected gene/protein is delivered into the cells. An example of this dominant-negative effect is Marfan syndrome, caused by a mutation in the fibrillin (FBNI) gene, in which the presence of a population of abnormal molecules could alter the entire fibril structure (Eldadah et al., 1995). The expression of mutant FBNI cDNA in normal human or mouse cells replicates the effects seen in cells from individuals with a single mutant allele and a normal allele, suggesting that the dominant-negative effect does explain the phenotype (Eldadah et al., 1995).

Infertility is a major public health problem that affects millions of couples (PA-040049, NIH 1-8-0004). Currently, patients with ovarian failure have no effective treatment available and are offered only supportive treatment. If women with ovarian failure desire to become pregnant, their only option is the controversial process of egg donation (Wentz, 1996). In many cases, this procedure is prohibitively expensive and ethically unacceptable. Egg donation also exposes both the recipient and the donor to considerable medical risks, such as ovarian hyperstimulation and complications of oocyte retrieval (Speroff et al., 1994). Even when the process is successful, the resulting child is not genetically related to the patient (Speroff et al., 1994).

The objective of our study was to develop a gene therapy approach to treat this type of infertility. This approach will significantly improve the reproductive health of women and provide treatment options for infertility due to gonadal failure, a condition, that is, currently considered incurable. Further work is currently underway in our laboratory to confirm our 
in vitro
 data 
in vivo
 in the FSHR knockout mice [follitropin-FSH receptor knockout mice (FORKO)], an animal model for ROS, that is, anovulatory and infertile (Dierich et al., 1998). Clearly several delivery obstacles have to be overcome. Also, a potential immune response to the adenovirus might create an inflammatory response within the ovary. We have accomplished intraovarian injection of adenovirus into numerous mice; the process was uncomplicated, and there was no evidence of hemorrhage, subsequent adhesion formation or inflammation (Al-Hendy et al., 2005). Our preliminary 
in vivo
 data with Ad-LacZ was reassuring, in that intraovarian-delivered adenovirus was not associated with systemic viral dissemination (Al-Hendy et al., 2005). However, the effect of such treatment on folliculogenesis and ovulation is not currently clear, and no effect of Ad-LacZ ovarian delivery on fecundity or pregnancy outcome in mice has been observed (Rhee et al., 2004; Al-Hendy et al., 2005). Another potential concern is the possibility of germine transmission of viral genes via the oocyte DNA. Again, when Ad-LacZ was delivered directly to the ovary, no adenoviral or LacZ gene, or gene products were detected in resulting pups, which suggests no germline transmission (Rhee et al., 2004; Al-Hendy et al., 2005). If our approach of adenovirus-mediated delivery of normal hFSHR is successful in supporting ovulation in models of primary ovarian failure, similar gene therapy approaches could be applied to other types of infertility that currently have limited treatment options.

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