Inhibin: a candidate gene for premature ovarian failure

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Premature ovarian failure (POF) occurs in 1% of all women, and in 0.1% of women under the age of 30 years. The mechanisms that give rise to POF are largely unknown. Inhibin has a role in regulating the pituitary secretion of FSH, and is therefore a potential candidate gene for ovarian failure. Using single-stranded conformation polymorphism (SSCP) and DNA sequencing, DNA samples were screened from 43 women with POF for mutations in the three inhibin genes. Two variants were found: a 1032C→T transition in the INHβA gene in one patient, and a 769G→A transition in the INHα gene in three patients. The INHβA variant appears to be a polymorphism, as there was no change in the amino acid sequence of the gene product. The INHα variant resulted in a non-conservative amino acid change, with a substitution from alanine to threonine. This alanine is highly conserved across species, and has the potential to affect receptor binding. The INHα variant is significantly associated with POF (3/43 patients; 7%) compared with control samples (1/150 normal controls; 0.7%) (Fisher's exact test, P < 0.035). Further analysis of the inhibin gene in POF patients and matched controls will determine its role in the aetiology of POF.

Key words: infertility/inhibin/mutation detection/ovarian failure/premature

Introduction

Premature ovarian failure (POF) is a condition causing amenorrhea, infertility, hypo-oestrogenism and elevated gonadotrophin concentrations in women under the age of 40 years. It is a common condition that occurs in 1% of all women, and in 0.1% of women before the age of 30 years (Coulam et al., 1986). Two significant consequences of POF are the loss of fertility, and the clinical effects of hypo-oestrogenism. Intermittent ovarian function has been reported in some women, and pregnancy can occur in ~5–10% of patients subsequent to diagnosis (Kalantaridou et al., 1998). Low concentrations of oestrogen from a young age appear to increase the risk of osteoporosis and coronary heart disease (Conway, 1997).

The best known causes for ovarian failure are sex chromosome abnormalities such as Turner syndrome. In most patients with POF and normal female chromosome constitution, however, no cause can be identified (for recent reviews see Conway, 1997; Anasti, 1998; Kalantaridou et al., 1998; Shelling, 2000). Iatrogenic agents, such as chemotherapy or radiotherapy, are known to reduce follicle numbers and cause POF. Although autoimmune diseases are seen in 10–20% of women with POF (Conway, 1997), the role of autoimmunity remains controversial in patients without other signs of autoimmune endocrine disease. Infections, such as mumps, have also been suggested to cause oophoritis resulting in ovarian failure. In a minority of cases a specific causative factor can be identified. These include galactosaemia, enzyme deficiencies and defects of gonadotrophin signalling. Approximately 5% of women with POF have a positive family history (Conway, 1997), suggesting an inherited predisposition to POF. Candidate genes or loci that have been suggested to cause either familial or sporadic POF include genes on the X chromosome (POF1, POF2, FMRI) and 3q22-3q23 in families with POF and blepharophimosis (recently reviewed by Shelling, 2000).

Most women with POF are found to have follicles, but they do not appear to respond to normal gonadotrophin stimulation (Conway, 1997). However, very few mutations have been identified in gonadotrophin hormones or their receptors. The loss of function mutation, 566C→T, identified in the FSH receptor (Aittomaki et al., 1995) was found to cause ovarian failure with amenorrhea in a group of Finnish families. It appears that this FSH receptor mutation is rare elsewhere, as it has not been detected in other populations (Conway, 1997; Layman et al., 1998). Ovarian resistance has also been seen in association with a premature stop codon in the LH receptor gene (Latronico et al., 1996). These data demonstrate the obvious importance of the FSH axis in ovarian function, and the possibility that abnormalities in the FSH response genes may be candidates for POF.

The glycoprotein, inhibin, is a potential candidate for POF due to its role in the negative feedback control of FSH, which has a pivotal role in the recruitment and development of ovarian follicles during folliculogenesis. Inhibin is structurally related to the transforming growth factor (TGF)-β superfamily,
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a group of multi-functional growth and differentiation factors. The mature inhibin is a 31–32 kDa heterodimeric glycoprotein consisting of an 18 kDa α-subunit linked by two disulphide bonds to one of two 14 kDa β-subunits (Vale et al., 1988). There are two forms of inhibin; inhibin A (α-βA), and inhibin B (α-βB). The homodimer of the β-subunit is the glycoprotein activin, which has an opposing function to inhibin. The inhibin subunits are encoded by three separate genes: INHA, INHβA and INHβB, which map to 2q33-pter, 2cen-q13 and 7p15-p14 respectively (Barton et al., 1989).

The main function of inhibin in women is its regulation of pituitary FSH secretion. A decline in serum inhibin concentrations has also been shown to occur when the ovarian follicular reservoir begins to subside (MacNaughton et al., 1992). Recent evidence suggests that changes in inhibin secretion are responsible for an increase in FSH, and may be a marker of ovarian reserve (Burger et al., 1998, Hofmann et al., 1998; Reame et al., 1998). It has been demonstrated that an increase in FSH secretion coincides with an increased rate of follicular depletion during the menopausal transition (Richardson et al., 1987).

The hormonal patterns of POF patients also implicate inhibin as being causative in the disease mechanism. A defect in inhibin secretion has been reported in women with POF (Pampfer and Thomas, 1989), and inhibin concentrations were lower in women with ovarian failure during both anovulatory and ovulatory rebound cycles compared with infertile women with normal ovulatory cycles (Buckler et al., 1991). It has also been noted that women with impending POF had lower follicular and luteal phase inhibin concentrations (Halvorson and Decherney, 1996).

It is proposed that a functional mutation in any of the inhibin genes would lead to a decrease in the amount of bioactive inhibin. This loss would result in an increase in the concentrations of FSH by removing the negative feedback to the pituitary, leading to premature depletion of follicles, and hence result in POF. We show that a 769G→A transition in the INHA gene occurs in ~7% of POF patients, and is associated with POF at a very early age.

**Materials and methods**

**Patient information**

A total of 43 women with POF was recruited for study in the Departments of Obstetrics and Gynaecology in Auckland, New Zealand, and Ljubljana, Slovenia. For the purpose of this study, POF was defined as cessation of menses for a duration of 6 months or longer before the age of 40 years, and a FSH concentration of >40 IU/l. All patients underwent a clinical assessment that included taking a complete medical and gynaecological history, and recording age at menses, menstrual history, and age at menopause. A serum gonadotrophin assessment was performed, and all patients were analysed for cytogenetic abnormalities. Five patients from the New Zealand samples had a family history of POF, defined as having more than one affected primary relative. Normal control samples were anonymous DNA samples from the general population, and would include males and females of various ages.

**DNA extraction**

Genomic DNA was extracted from 10 ml samples of blood. Lymphocytes were isolated from blood samples using the NYCOMED Lymphoprep™ kit (Nycomed, Oslo, Norway). Cells were incubated at 65°C for 1 h with 3.5 ml guanidine hydrochloride solution (6 mol/l), 250 µl ammonium acetate solution (7.5 mol/l), 50 µl proteinase K solution (10 mg/ml) and 250 µl sodium sarcosyl solution (20% w/v). Cells were added to 2 ml of cold chloroform and then centrifuged at 2500 g for 3 min. The top layer was collected, and 10 ml of cold absolute ethanol added to precipitate the DNA. DNA was stored in 200 µl Tris–EDTA buffer at 4°C.

**Polymerase chain reaction (PCR)**

PCR primers were designed spanning the mature peptides of each inhibin gene, i.e. for INHA (nucleotides 841–1242) (Mayo et al., 1986), INHβA (nucleotides 1167–1528) (Mason et al., 1986) and INHβB (nucleotides 717–1061) (Mason et al., 1986) using the Primer Select module in the DNASTAR computer program from Lasergene 1994 (DNASTAR Inc., Madison, WI, USA). Primers flanking the whole region were designed to give one large fragment for each gene, which was used for DNA sequencing. Smaller overlapping fragments of 200–300 bp were designed that spanned the functional region and were used for single-stranded conformation polymorphism (SSCP) analysis. The primers flanking each fragment are shown in Table I. The primers were dissolved in sterile water to give a final concentration of 20 mol/l.

**PCR conditions**

PCR was carried out using Taq DNA polymerase (Qiagen GmbH, Hilden, Germany) and PCR buffer. Genomic DNA (100 ng) was amplified in a 25 µl volume reaction containing 2.5 µl of PCR buffer (1 ×), 25 nmol of each dNTP, 5 nmol of forward and reverse primers, and 0.125 µl Taq DNA polymerase. β-Globin was used as positive control, and a nil DNA reaction was used as a negative control for all PCR reactions. Standard PCR conditions comprised 94°C denaturation for 1 min, 58°C annealing for 1 min, and 72°C extension for 1 min for 30 cycles. To ensure that a single band of expected size was present after amplification, electrophoresis of 5 µl of each PCR product was carried out in a 1.5% agarose gel and visualized under UV light using an ethidium bromide stain.

**Single-stranded conformation polymorphism (SSCP)**

An initial group of samples from 12 patients was analysed by SSCP analysis, to determine whether this was a suitable mutation detection strategy. The remaining 31 samples were analysed by DNA sequencing only. The PCR products were diluted 1/10 with sterile water. Equal volumes of diluted sample and 2 × formamide loading buffer were heated to 95°C for 3 min to denature the samples, and immediately placed on ice to prevent DNA strands from re-annealing. A 3 µl aliquot of each sample was electrophoresed alongside non-denatured and denatured controls. The SSCP gels consisted of 1 × Tris–borate–EDTA (TBE) buffer, 8% or 10% polyacrylamide, with or without glycercol (5%). Setting agents were 15 µl (25% w/v) ammonium persulphate and 15 µl N,N,N′,N′-tetramethylethylenediamine (TEMED) for every 10 ml of non-denaturing gel. Electrophoresis was performed at room temperature (20–24°C), using 0.5 × TBE running buffer. Mini gels (BioRad mini-protein II cell; BioRad, Hercules, CA, USA) were electrophoresed for 2–3 h at 170 V and large gels (BioRad SequiGen Sequencing cell) were electrophoresed overnight at 200–300 V. The DNA was visualized using silver staining. Gels were fixed in equal volumes of 40% ethanol and 10% acetic acid for a least 30 min, followed by two 15 min washes in a second mixture of 10% ethanol and 5% acetic acid. Fixation was followed by a 15 min wash in a K2Cr2O7-based oxidizer. Gels were then washed in distilled water until the yellow coloration of the oxidizer was completely removed. The gels were then stained in...
Table 1. Primers for mutation detection and sequencing. Primers (forward and reverse) flanking each fragment in the three inhibin genes. The size of the PCR products generated by each set of primers is indicated. The location of the primers is shown with reference to corresponding nucleotides in the corresponding genes. PCR primers were designed spanning the mature peptides of each gene for \textit{INH}α (nucleotides 841–1242) (Mayo et al., 1986), \textit{INH}βA (nucleotides 1167–1528) (Mason et al., 1986) and \textit{INH}βB (nucleotides 717–1061) (Mason et al., 1986) using the Primer Select module in the DNASTar computer program from Lasergene 1994 (DNASTAR Inc., Madison, WI, USA).

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Size (bp)</th>
<th>Primers (5’ to 3’)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Inhibin alpha}</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>\textit{INH}α</td>
<td>601</td>
<td>Forward GCTGCTGCGCTGTCCCCTCTGA 373...754</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse TATTTCCCCAACTCTGGCTTTTCCTC 1323...1309</td>
<td></td>
</tr>
<tr>
<td>\textit{INH}α1</td>
<td>243</td>
<td>Forward GGCCCAACCTCGGACCAGAC 792...811</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse AGCCCAACACACCATTACAGTAG 1034...1011</td>
<td></td>
</tr>
<tr>
<td>\textit{INH}α2</td>
<td>139</td>
<td>Forward GCTGCGCTGAGGACCGTGGAT 963...983</td>
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<tr>
<td></td>
<td></td>
<td>Reverse GGAGTGGGGCTGGGGCTGGTGG 1101...1078</td>
<td></td>
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<tr>
<td>\textit{INH}α3</td>
<td>254</td>
<td>Forward CTACCCAGGCCAGCCTACTCCTC 1079...1102</td>
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<td></td>
<td></td>
<td>Reverse TATTTCCCCAACTCTGGCTTTTCCTC 1332...1309</td>
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<tr>
<td>\textit{Inhibin beta A}</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>\textit{INH}βA</td>
<td>529</td>
<td>Forward CTTGGGCGAAGAAGAGGAAGAAAGAA 1005...1028</td>
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<tr>
<td></td>
<td></td>
<td>Reverse CCTGCCCCGCTGGCAACTC 1533...1517</td>
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<tr>
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<td>Forward GCAGAAGAAGAAGAAAGGAGGAA 1071...1094</td>
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<td>\textit{INH}βA2</td>
<td>268</td>
<td>Forward GGAGCAGCCGGTCTCCACTG 1314...1334</td>
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<td></td>
<td></td>
<td>Reverse TCTCTCTTGGCCAGCTGCTCTTC 1581...1558</td>
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<tr>
<td>\textit{Inhibin beta B}</td>
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<tr>
<td>\textit{INH}βB</td>
<td>586</td>
<td>Forward CGTGGGCTGCGGCTGTCGGAC 617...638</td>
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<td></td>
<td></td>
<td>Reverse CTGCCAGCCCAACACAGATGC 1185...1163</td>
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<tr>
<td>\textit{INH}βB1</td>
<td>202</td>
<td>Forward CGTGGGTTCGGGTGGTCTGGAC 617...638</td>
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<td></td>
<td></td>
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<tr>
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<td>Forward GCACCCAGCCGCTACTACG 807...825</td>
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<td></td>
<td></td>
<td>Reverse TTCCGCTGGCATGCTGAGTTG 1024...1006</td>
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<tr>
<td>\textit{INH}βB3</td>
<td>241</td>
<td>Forward AAACCTGCTGCTACCTCCACAA 945...967</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Reverse CTCCTGAGCCCAACACAGAATGACT 1185...1163</td>
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20 ml of silver reagent dissolved in 180 ml of distilled water for 20 min. Development of the gels was performed by washing in distilled water for 1 min, followed by three washes in 200 ml aliquots of developer, and a final 5 min wash in 5% acetic acid. The gels were washed to remove the acetic acid, transferred to filter paper (3 MM; Whatman, Clifton, NJ, USA), dried and stored.

**DNA sequencing**

All 43 samples were analysed by DNA sequencing. The large PCR fragments, \textit{INH}α (601 bp), \textit{INH}βA (529 bp) and \textit{INH}βB (586 bp), that spanned the entire functional region of each of the three inhibin genes (Table 1), were used as templates for DNA sequencing. Samples were purified with Promega’s Wizard PCR Purification System (Promega Corp., Madison, WI, USA), and sequencing was performed using an Applied Biosystems Model 377 automated sequencer (PE Biosystems, Foster City, CA, USA) and 2 µl of template DNA.

**Characterization of variants**

The PCR products from ethnically matched control samples were analysed for the variant 769G→A in the \textit{INH}α gene subunit. Restriction fragment length polymorphism (RFLP) analysis used \textit{Bst}I11 as the restriction enzyme to determine if this variant was a naturally occurring polymorphism, or a mutation that may be responsible for POF. DNA with a known sequence was used as negative control DNA, while DNA from a patient shown by sequence analysis to be a carrier of the variant 769G→A acted as a positive control for the RFLP test. The restriction enzyme digest was undertaken in 1× restriction buffer using 2.5 U of \textit{Bst}I11, 0.2 µl of acetylated bovine serum albumin (BSA), 5 µl of PCR product, and sterile water to give a total reaction volume of 20 µl. The reaction mixtures were incubated at 37°C for 1–2 h, electrophoresed in a 2% agarose gel, and stained with ethidium bromide. To allow better separation of the fragments, some of the samples were also electrophoresed in an 8% polyacrylamide gel and subsequently stained with SYBR Gold (Molecular Probes Inc., Eugene, OR, USA). The undigested DNA control was also incubated in a reaction mixture containing all the above reagents except the \textit{Bst}I11 enzyme, and electrophoresed alongside the digested PCR products. The wild-type \textit{INH}α1 PCR product yields three fragments of 85 bp, 25 bp and 134 bp when digested with \textit{Bst}I11. In the presence of the 769G→A variant, the enzyme recognition site CGTCG(N)12 is abolished, and hence yields only two fragments of 85 bp and 159 bp. A heterozygous sample will display all four fragments.

**Results**

Patients (\(n = 43\)) with POF were screened for mutations in the three inhibin genes. Two variants were detected in the \textit{INH}βA1 and the \textit{INH}α1 fragments using SSCP analysis in the initial group of 12 unrelated New Zealand POF patients (data not shown). The fragments \textit{INH}α2, \textit{INH}α3, \textit{INH}βA2, \textit{INH}βA1, \textit{INH}βB1, \textit{INH}βB2 and \textit{INH}βB3 did not reveal any migration variants in any of the patient samples when compared against the wild-type DNA.

The migrational shift detected in the \textit{INH}βA1 fragment was caused by a silent substitution at nucleotide 1032C→T (Figure 1a). This variant did not change the amino acid sequence of the inhibin beta A subunit as it occurred in the third position of the codon, causing a GGC (glycine) to GGT (glycine) alteration.
Direct DNA sequencing of the PCR product confirmed the \textit{INH\alpha}1 variant detected using SSCP. The variant was the result of a G→A missense substitution at nucleotide 769 (Figure 1b) that alters codon 257 from GCT to ACT, resulting in an alanine to threonine amino acid substitution in the \textit{INH\alpha} gene subunit. To confirm that the \textit{INH\alpha} 769G→A variant was not a sequencing error, the \textit{INH\alpha}1 fragment was amplified from both the original DNA sample, DNA extracted from a second blood sample, and each was sequenced in both directions. Again, the same sequence variation was identified. Finally, sequencing was performed on the \textit{INH\alpha} (601 bp), \textit{INH\beta}A (529 bp) and \textit{INH\beta}B (586 bp) amplification products from all 12 patients, and no additional variants were found.

In order to extend the above study, DNA was collected from a further six affected women with POF from New Zealand, and 25 from Slovenia. DNA was also collected from seven women with primary amenorrhea (from Slovenia). The fragments \textit{INH\alpha} (601 bp), \textit{INH\beta}A (529 bp) and \textit{INH\beta}B (586 bp) were amplified from these 31 POF patients and seven primary amenorrhea patients, and each was sequenced. Another two patients were found to carry the \textit{INH\alpha} 769G→A variant. No other sequence variants were identified in any of the POF patients, nor from DNA obtained from the seven women with primary amenorrhea.

A rapid RFLP screen was developed to identify the presence of the \textit{INH\alpha} 769G→A variant. This variant abolished a \textit{Bst}I restriction enzyme site. An RFLP analysis of the 244 bp \textit{INH\alpha} amplification products from DNA samples of 150 ethnically matched normal individuals (100 from New Zealand and 50 from Slovenia) showed only 134 bp and 85 bp fragments, except in a single sample which showed heterozygosity. The patients with the 769G→A variant demonstrated heterozygosity with fragment lengths of 159 bp, 134 bp and 85 bp (Figure 2). According to Fisher’s exact test, the likelihood of finding a variant in the POF patient samples (3/43; 7%) was significantly different from finding a variant in a group of matched controls (1/150; 0.7%) (\(P < 0.035\)).

\section*{Discussion}

POF is a condition affecting 1% of women under the age of 40 years (Coulam \textit{et al}, 1986), with most of the cases being idiopathic (Conway, 1997). POF can result from a defect in either the development or function of the ovaries. We suggest that mutations in one of the inhibin genes allow a period of normal ovarian function before the onset of early POF.

The 1032C→T transition in the \textit{INH\beta}A gene (Figure 1a) identified in one of 12 New Zealand patients was not found to change the amino acid sequence of the polypeptide product, and is most likely a synonymous substitution creating a silent polymorphism. However, this particular nucleotide is conserved in porcine, bovine and human \textit{INH\beta}A genes (Mason \textit{et al}, 1986), and this patient had menopause at age 25 years.

The 769G→A variant was initially identified in the \textit{INH\alpha} gene (Figure 1b) in a single New Zealand patient by SSCP. This results in the substitution of alanine for threonine at codon 257. A further two probands were subsequently identified by DNA sequencing to be heterozygous for this variant. RFLP analysis suggests that the \textit{INH\alpha} 769G→A variant is rare in
The functional significance of the amino acid variant at codon 257 is unknown. Analysis of the protein structure suggests that it could interfere with receptor binding. The inhibin molecule is part of the TGF-β superfamily, a group of multi-functional growth and differentiation factors. Overall, this family consists of ~30 members that show a conserved carboxyl terminal 7-cysteine domain with between 20 and 92% sequence identity (Griffith et al., 1996). The three-dimensional crystal structure of INHα is unknown; however, the structure of some other members of the TGF-β superfamily has been determined. These are TGF-β2 and OP-1 (oestrogenic protein-1), with an amino acid sequence homology to INHα of 24 and 26% respectively (Griffith et al., 1996). INHα, like the other members of the TGF-β superfamily, has the seven conserved cysteines. However, the amino-terminal region upstream of the first cysteine is divergent from other members of the TGF-β superfamily, and is thought to be involved in receptor binding (Griffith et al., 1996). The alanine to threonine change occurs within this putative receptor binding region of the final mature peptide. We suggest that this change would be sufficient to impair the binding of inhibin to its receptor resulting in an inability to activate the subsequent signal transduction pathway. Alternative hypotheses for a functional effect of this transition mutation include preventing dimer formation, cleavage of the mature peptide, or altering glycosylation.

The parents of one of the affected individuals carrying the 769G→A variant were able to provide DNA samples to determine whether the INHα variant was inherited or was a de-novo mutation. The father showed a normal DNA sequence, while the mother was found to be a carrier for the variant. It is interesting to note that she had a normal menopause at age 55 years. It might be expected in reproductive disorders that highly penetrant mutations causing infertility would not be common, and would be rapidly lost from the gene pool. Therefore, it would be expected to see incomplete penetrance in familial POF, and evidence was recently provided for the dominant transmission of POF with 79.1% penetrance in a study of 71 women (Vegetti et al., 1998).

The only way to be certain that a mutation causes a functional effect is to perform functional testing. Most functional studies involve the expression of receptors in a recombinant expression system accompanied by an assay with the protein of interest. However, the inhibin receptor has yet to be characterized. The activity of inhibin is currently investigated by an in-vitro bioassay based on the effect of graded doses of inhibin on FSH cell content of rat pituitary cells in culture (Robertson and de Kretser, 1989). The identification of this INHα variant highlights the importance of the inhibin glycoproteins in the normal function of the female reproductive system. Women with the INHα variant had relatively severe symptoms of POF with secondary amenorrhoea at the age of 16, 20 and 24 years (Figure 4). It is interesting that none of the seven patients with primary amenorrhoea was found to be a carrier of the variant, which would suggest that mutations in inhibin are not a common cause of primary amenorrhoea. The three women with the INHα variant represented 7% of the study group of 43. If this were an accurate indication of the number of cases of POF caused by this mutation, then it would affect a substantial portion of young POF patients (25% of patients under the age

| Human | PWSPGALRLQRPPEPEPAHANC | Horse | PWSPGALRLQRPPEPEPAHANC | Porcine | PWSPGALRLQRPPEPAHAVAC | Ovine | PWSPGALRLQRPPEPEPAHANC | Mouse | PWSPGALRLQRPPEPAHANC | Bovine | PWSPGALRLQRPPEPEPAHANC | Possum | PWSPGALRLQRPPEPAHANC | Chicken | PWSPGALRLQRPPEPEPAHANC | Rat | PWSPGALRLQRPPEPAHANC |

Figure 3. Alignment of the INHα gene subunit amino acid sequences from human, horse, porcine, ovine, mouse, bovine, possum, chicken and rat species. DNA sequences were obtained from Genbank. The arrow indicates the amino acid altered by the G→A mutation.

Figure 4. Age at menopause. The age at menopause was obtained from each patient, and the distribution was plotted according to this age. The three women carrying the 769G→A variant are indicated by the solid circles.
of 25 years). Functional and further population studies of POF patients, with ethnically matched controls, are required to determine the exact contribution of this variant to POF. A genetic test, based on the Bsr711 restriction analysis, could prove to be an important diagnostic tool. Detection of the mutation prior to the development of ovarian failure enables carriers to make informed decisions regarding reproductive options. Early detection would provide better opportunity for early intervention such as replacement of the inhibin hormone in patients to delay the onset of ovarian failure.

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