Identifying Genetic Risk Factors for Low Ovarian Response

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Abstract

Background: One of the most frustrating problems in the treatment of infertility is that of poor ovarian response to stimulation. It would therefore be advantageous to have a genetic marker that could predict low ovarian reserve at a young age before the impact of low ovarian reserve affects a woman’s fertility. Since both BMP15 and GDF9 gene mutations have been reported in women with premature ovarian failure, the purpose of the present study is to investigate the correlation between low ovarian reserve BMP15 c. A704G (p. Y235C), BMP15 c.T443C (p.L148P), GDF9 c.C557A (p. S186Y), and GDF9 c. A199C (K67E) polymorphisms.

Methods: Buccal swabs were obtained from 90 women, of whom 50 had a diagnosis of low ovarian reserve and 40 were fertile controls. DNA was extracted from the buccal swabs followed by polymerase chain reaction-sequence based typing (PCR-SBT) amplification to examine the frequencies of BMP15 Y235C, BMP15 L148P, GDF9 S186Y, and GDF9 K67E polymorphisms.

Results: No significant differences were observed when the frequencies between BMP15 Y235C, BMP15 L148P, GDF9 S186Y, and GDF9 K67E polymorphisms among women with a diagnosis of low ovarian reserve were compared with those of control women.

Conclusion: BMP15 Y235C, BMP15 L148P, GDF9 S186Y, and GDF9 K67E polymorphisms cannot be used as markers of low ovarian reserve.

Keywords: BMP15 Y235C; BMP15 L148P; GDF9 S186Y; and GDF9 K67E polymorphisms; low ovarian reserve

Introduction

One of the most frustrating problems in IVF today is that of poor ovarian response. This event has been called poor ovarian reserve, low ovarian reserve, diminished ovarian reserve, premature ovarian aging, and premature ovarian insufficiency. The number of oocytes retrieved directly influences success rates after in vitro fertilization (IVF) and embryo transfer (ET) [1]. Poor ovarian response reduces the number of embryos generated and results in decreased pregnancy rates in both index and subsequent IVF cycles [2]. Decreased pregnancy rates per cycle of IVF lead to more cycles being performed in an attempt to accomplish the goal of desired family. Poor response to gonadotropin is a significant problem in assisted conception occurring in 9% to 24% of patients and can precede the diagnosis of premature ovarian failure by months to years [3]. Thus, the economic impact of low ovarian reserve is significant. Identification of women with low ovarian reserve would allow consideration of more cost efficient management of infertile patients. Genetic markers are needed so that young women predisposed to low ovarian reserve could be informed and exercise reproductive options early. While antimullerian hormone (AMH) serum concentrations remain an appropriate marker of low
ovarian reserve, no significant differences in polymorphisms of the AMH gene and its receptor AMH R1 were found when infertile women undergoing IVF with the diagnosis of low ovarian reserve were compared with fertile women [4]. Recent animal studies indicate oocyte-specific genes play important roles in regulation of oogenesis and folliculogenesis [5]. Genetic studies in mice demonstrated critical roles of two key oocyte-derived growth factors belonging to the transforming growth factor-β (TGF-β) superfamily, growth and differentiation factor-9 (GDF-9) and bone morphogenetic protein-15 (BMP-15), in ovarian function [6,7]. The BMP-15 gene, also named GD9B, is encoded by two exons and maps to the X chromosome in Xp11.2 [8]. The GDF9 gene is also encoded by two exons and maps to chromosome 5 in 5q23.3 [9]. The identification of BMP15 and GDF9 gene mutations as the causal mechanism underlying infertility in several sheep strains in a dosage-sensitive manner also highlighted the crucial role these two genes play in ovarian function [10-12]. Both BMP15 and GDF9 gene mutations have been reported in women with premature ovarian failure [13-20]. The purpose of the current study is to evaluate the association of BMP15 and GDF9 gene mutations in women with premature ovarian failure [13-20]. The frequencies of BMP15 Y235C, BMP15 L148P, GDF9 S186Y, and GDF9 K67E polymorphisms among women who had a diagnosis of low ovarian reserve were compared with fertile control women and are shown in Table 2. Only those of fertile control women and are shown in Table 2. Only

Materials and Methods

Patients

Ninety women, of which 50 had a diagnosis of low ovarian reserve (LOR) and 40 are fertile controls, were included in the study. All LOR patients experienced regular menstrual cycles (mean 25-35 days), underwent IVF for treatment of infertility and had no relevant systemic disease, severe endometriosis, nor uterine or ovarian abnormalities. A diagnosis of low ovarian reserve was based on secondary amenorrhea for >6 months in women <40 years of age, FSH >20 mIU/ml, and no antral follicles on transvaginal scanning, and/or serum AMH concentrations less than 1.05 ng/ml [21]. Forty fertile women age 40 years or older who had a history of menstruating after age 40 years and no history of infertility constitute the control group. The ethnicity of the patients and controls were mixed with 30% of Arabic descents living in Cairo, Egypt and 50% Caucasians living in midwestern United States of America and equal numbers with patients and controls.

This study was approved by the FC Lab institutional review board (IRB). All the couples and control women gave written informed consent.

DNA Extraction

All women had their cheeks swabbed with a cotton or Dacron swab to collect cells for DNA analysis. DNA was extracted from buccal swabs using a Promega Maxwell 16 instrument, with the Promega DNA IQ

Casework Pro Kit. All procedures are performed according to the manufacturer's instructions. Purified DNA was eluted into a final volume of 30 μL 10 mM Tris, 0.1mM EDTA, pH 8.

Polymerase Chain Reaction

DNA samples were first mixed with the appropriate reagents for the desired polymerase chain reaction assay, including oligodeoxynucleotide primers specific for each mutation site being analyzed. Thermal cycling was conducted in a GeneMate Genius Thermal Cycler per the manufacturer's instructions, using 0.2 mL PCR tubes (Axygen). Following PCR, the PCR products were purified using a Qiagen QIAquick PCR Purification Kit or Invitrogen PCR Micro Kit and the DNA sequenced to reveal the mutation regions. DNA sequencing was performed by NUSeq Core Facility, which is supported by the Northwestern University Center for Genetic Medicine, Feinberg School of Medicine, and Shared and Core Facilities of the University's Office for Research. The primer sequences are listed in Table 1. PCR cycles and stages are:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Tm (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP15 L148P (F)</td>
<td>5’-TACCTGGCATATACAGATCCT-3’</td>
<td>58.7</td>
</tr>
<tr>
<td>BMP15 L148P (R)</td>
<td>5’TGAACAGTTGTGGATACCAT-3’</td>
<td>57.4</td>
</tr>
<tr>
<td>BMP15 Y235C (F)</td>
<td>5’-TCGCAAGGGTCTGGAATA-3’</td>
<td>56.3</td>
</tr>
<tr>
<td>BMP15 Y235C (R)</td>
<td>5’-AACCTCAGCTGAGATACCA-3’</td>
<td>58.0</td>
</tr>
<tr>
<td>GDF9 L67G (F)</td>
<td>5’TATGCTTTGGTCTCAGGCT-3’</td>
<td>60.4</td>
</tr>
<tr>
<td>GDF9 L67G (R)</td>
<td>5’-AGTTGTTGAGGTTGACTCTAT-3’</td>
<td>59.2</td>
</tr>
<tr>
<td>GDF9 S186Y (F)</td>
<td>5’TACTACGTTGAACACTTACTCA-3’</td>
<td>59.2</td>
</tr>
<tr>
<td>GDF9 S186Y (R)</td>
<td>5’-TATGAACTGGTAGATAGACCTTGTG-3’</td>
<td>58.3</td>
</tr>
</tbody>
</table>

Table 1. The primer sequences for of BMP15 Y235C, BMP15 L148P, GDF9 S186Y, and GDF9 L67G polymorphisms.

Statistical Analysis

The frequencies of BMP15 Y235C [13], BMP15 L148P [16], GDF9 S186Y [16], and GDF9 K67E [15] polymorphisms among women with a diagnosis of LOR were compared with controls using ANOVA one way analysis of variance. Significance is defined as p<0.05.

Results

The frequencies of BMP15 Y235C, BMP15 L148P, GDF9 S186Y, and GDF9 K67E polymorphisms among women who had a diagnosis of low ovarian reserve were compared with those of fertile control women and are shown in Table 2. Only
One heterozygous BMP-15 L148P mutation was found among the control women. She was a 61 year old Gravida 3 Para 2 with one miscarriage. She underwent menopause at the age of 50 years. No mutations were found among women who had a diagnosis of low ovarian reserve.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Control</th>
<th>LOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP15 L148P</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild Type</td>
<td>40 (98%)</td>
<td>50</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Homozygous</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>BMP15 Y235C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild Type</td>
<td>40 (100%)</td>
<td>50</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Homozygous</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>GDF9 S186Y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild Type</td>
<td>40 (100%)</td>
<td>50</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Homozygous</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>GDF9 L76G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild Type</td>
<td>40 (100%)</td>
<td>50</td>
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<tr>
<td>Heterozygous</td>
<td>0 (0%)</td>
<td>0</td>
</tr>
<tr>
<td>Homozygous</td>
<td>0 (0%)</td>
<td>0</td>
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</tbody>
</table>

Table 2. Frequencies of BMP15 Y235C, BMP15 L148P, GDF9 S186Y and GDF9 L67G polymorphisms among women who had a diagnosis of low ovarian reserve (LOR) compared with those of fertile control women.

**Discussion**

Low ovarian reserve is a decrease in the quantity or quality of oocytes, leading to impaired fertility. It results from the depletion of a finite ovarian follicle pool that is produced during embryonic development. Although X chromosome abnormalities such as monosomy X (Turner’s syndrome) and increased numbers of CGG repeats have been associated with low ovarian reserve, genetically predetermined loss of oocytes is thought to be the most common cause of diminished ovarian reserve [22]. The germ cells which form this ovarian reserve undergo a process that involves the coordination of meiotic progression and the breakdown of germ cell cysts into individual oocytes housed within primordial follicles [23]. Recent evidence indicates that genetic factors including BMP-15 and GDF-9 can specifically perturb primordial follicle assembly [19]. The BMP-15 gene, also named GDF9B, is encoded by two exons and maps to chromosome Xp11.2 [7]. The GDF9 gene is also encoded by two exons and maps to chromosome 5 in 5q23.3 [8].

Even though genetic studies in mice [5,6], sheep [9-12] and humans [13-20] demonstrated critical roles of GDF-9 and BMP-15 in ovarian function, no mutations in BMP15 Y235C [13], BMP15 L148P [16], GDF 9 S186Y [16], and GDF9 K67E were observed among women with a diagnosis of low ovarian reserve. This finding could reflect the prevalence of these polymorphisms in the population [16]. While the frequency of mutations in BMP15 and GDF9 in the general population is not known, a point mutation in BMP15 has been associated with premature ovarian failure [13]. A 4bp deletion of GDF9 apparently leading to a premature stop codon has been reported in dizygous twins from one family [14] and two missense mutations in GDF9 have been identified in an individual with premature ovarian failure [15].

The goal of the current study was to identify a genetic marker for women at risk for future low ovarian reserve so that young women predisposed to low ovarian reserve could be informed and exercise reproductive options early including having their family first and their career second or oocyte cryopreservation [24]. While AMH serum concentrations remain an appropriate marker of low ovarian reserve, no significant differences in polymorphisms of the AMH gene and its receptor AMH RII were found when infertile women undergoing IVF with the diagnosis of low ovarian reserve were compared with fertile women [4]. Results of the present study suggest BMP15 and GDF9 cannot be used as practical markers for identifying women at risk for low ovarian reserve. Ovarian oogenesis and folliculogenesis are complex and coordinated processes. Future attempts at identifying genetic markers for low ovarian reserve should consider using methods in addition to traditional DNA sequencing, such as Next Generation Sequencing and Microarray analysis that allow much larger amounts of information to be generated very rapidly.

**Acknowledgements**

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**References**


