Single Nucleotide Polymorphisms of BMP15 are Associated with Poor Ovarian Response in In Vitro Fertilization Programs

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Abstract

Background: Poor ovarian response represents a major negative contribution to the efficacy of In vitro fertilization programs. It has been shown that specific nucleotide sequence variants of BMP15 gene, which encodes the growth factor specifically expressed by oocytes to regulate follicle development, are related to certain forms of ovarian dysfunction. The aim of the study was to search for novel variants related to poor ovarian response phenotypes by means of exon sequencing, and also to check possible relations of already known BMP15 variants to this particular form of ovarian insufficiency.

Methods: A total of 150 patients (65 women with poor ovarian response and 85 women with normal ovarian response as a control group) participated in this retrospective case-control study. All patients received ovarian stimulation according to the protocol with follicle-stimulating hormone and gonadotropin-releasing hormone antagonist. Genotyping was carried out by polymerase chain reaction with consequent readout of melting curves by means of modified kissing probes assay. Statistical tests were two-sided, with percent values compared by χ² test and associations measured by odds ratio.

Results: Two novel single nucleotide polymorphisms of BMP15 were revealed by exon sequencing. Of these, the c.607 C>T substitution was found only in the control group. In contrast, the novel single nucleotide deletion c.-8 delC in the 5' non-coding region of BMP15 mRNA was significantly more common in the poor ovarian response group. For the previously known sequence variants, the statistical analysis revealed associations of poor ovarian response with two single nucleotide substitutions, the exonic c.308A>G and the intronic c.328+905A>G.

Conclusion: Two novel variants of BMP15, both of plausible clinical relevance, were found in this study. Examination of larger patient cohorts is required to further elucidate their connection with the phenomenon of poor ovarian response in In vitro fertilization programs.

Keywords: BMP15; In vitro fertilization; Poor ovarian response; Single nucleotide polymorphism

Abbreviations: BMP-15: Bone Morphogenetic Protein; POR: Poor Ovarian Response; ORT: Ovarian Reserve Test; AFC: Antral Follicle Counts; AMH: Anti-Mullerian Hormone; IVF: In Vitro Fertilization; SNP: Single Nucleotide Polymorphism(s); OD: Odds Ratio

Introduction

Poor ovarian response (POR) is a term denoting the reduced number of ultrasound-detectable growing follicles during gonadotropin stimulation (apart from specification of underlying functional condition, which represents a special issue). By European Society of Human Reproduction and Embryology consensus on POR definition (the Bologna criteria), at least two of the following three features must be present: (i) advanced maternal age (≥ 40 years) or any of the other specific risk factors, (ii) ≤ 3 oocytes retrieved using a conventional controlled ovarian stimulation protocol, and (iii) an abnormal ovarian reserve test (ORT); i.e., low antral follicle counts (AFC; less than 5–7) or low blood levels of anti-mullerian hormone (AMH; <0.5–1.1 ng/ml)). Thus, patients over 40 years of age with an abnormal ORT may be a priori classified as poor responders (or, more properly, the expected PORs); on the other hand, two episodes of poor response after maximal stimulation are considered sufficient to define a patient as a poor responder in the absence of advanced maternal age or abnormal ORT [1]. The prevalence of poor responders varies in the literature between 9 and 24%; POR is accounting for approx. 50% of total number of cancelled In vitro fertilization (IVF) cycles, and it is a major negative contribution to the efficacy of IVF programs [2].

The exact causes of POR are not very well understood, although its high prevalence probably correlates with the advanced maternal age. In younger women, POR is frequently conditioned by premature ovarian failure, a disease of complex epidemiology with up to 30% of the cases constituted by familial forms [3]. Associations between nucleotide sequence variation and the premature ovarian failure susceptibility have been studied for a number of genes [4]. Among these BMP15 occupies a special place because it was cloned as a marker for premature ovarian failure [5].

The gene encodes bone morphogenetic protein 15 (BMP-15), a growth factor of the TGF-β superfamily. Specific expression of BMP15 in oocytes begins on the early stages of follicle development. Forming complexes with another factor, GDF-9, the BMP-15 secretory proteins promote early follicle growth by enhancing granulosa cell proliferation, modulate hormone production by granulosa cells, and subsequently restrain dominant/pre-ovulatory follicle development by inhibiting the FSHR gene activity [6,7]. Expression of BMP15 in oocytes is critical for recruitment and growth of follicles, and alterations in its...
Results

No significant differences in morbidity or obstetrical history were observed between the groups; however, the patients of the POR group turned to be older than the controls (34.7 ± 3.9 and 31.9 ± 3.9, respectively, p<0.05; Table 1) and have significantly longer histories of infertility, as well as higher FSH levels and lower AMH levels. Ultrasound examinations revealed lower ovarian reserve (decreased ovarian volume and AFC ≤ 5–7) for most women of this group as compared to their control counterparts (Table 1).

Allele frequencies for seven BMP15 SNP are given in Table 2. Minor allele frequencies ranged from 0.007 to 0.626. Statistical analysis revealed an association of POR with the exonic SNP rs41308662 (c.308 A>G; the POR group was significantly enriched with the rare G allele: three cases of heterozygosity versus none in the control group, p<0.05; Table 3), and probably also with the intronic SNP rs3897937 (c.328+905 A>G; an increased proportion of the G allele was observed in the POR group: 0.62 versus 0.46 in the control group, p ≈ 0.05; Table 3), Combined frequency of c.308G and c.328+905G was 2.6 ± 0.1 times higher in POR group as compared to the control group, whereas frequencies of other previously known SNP did not differ significantly between the groups.

Two novel SNP of BMP15 were revealed by exon sequencing and submitted for registration in ClinVar database. These were c.607 C>T (it was assigned rs796052131) and c.-8 delC (assigned rs796052132). The C>T substitution was found only in the control group, thus it may associate with functional preservation of ovaries. In contrast,

Materials and Methods

A total of 150 patients (65 women with POR and 85 women with the normal ovarian response as a control group) participated in the retrospective case-control study. All of them were recruited while receiving IVF treatment in a hospital of the Research Center for Obstetrics, Gynecology and Perinatology between September 2012 and August 2014. Inclusion criteria were as follows: age under 40, normal karyotype, integrity of both ovaries, and no history of ovarian surgery or pelvic radiation therapy. Thus, we excluded the gross risk factors (age in the first place), so no one of the patients was subject to the 1° of the Bologna criteria. It is an important restriction because the gene we are dealing with is specifically expressed in growing oocytes and almost nowhere else, and this was done to bring to the forefront some pathologic processes in the ovary per se but not reflections of major organic disturbances. The written informed consent was obtained from all participants; the study was approved by the Institutional Review Board at the Research Center for Obstetrics, Gynecology and Perinatology.

Ovarian stimulation was commenced using recombinant follicle-stimulating hormone, FSH (Gonal-F up to 300 IU/day, Merck Serono SA, Geneva, Switzerland) from day 3 of the cycle, and gonadotropin-releasing hormone antagonist (Cetrotid 0.25 mg, Merck Serono SA) was started when at least 1 follicle of ≥ 14 mm could be observed by ultrasound. [16]. The AMH blood levels were measured using AMH Gen II ELISA kit (A79765, Beckman Coulter, Brea, CA, USA). The genotyping was carried out using Prep-GS-Genetics kits for DNA extraction and original labeled oligonucleotides for the modified kitting probe assay (all consumables were provided by DNA-Technology JSC, Moscow, Russia). DNA amplification, detection of fluorescence, and digital analysis of melting curves were done with DT-96 Real-Time PCR Cycler (DNA-Technology JSC).

The data were analyzed using Statistica 10 software (StatSoft, Tulsa, OK, USA); p-values <0.05 obtained by two-sided tests were considered significant. Categorical variables were converted into percent values and assessed by χ² test, while associations were measured by odds ratio within 95% confidence interval.
Table 3: Comparison of allele frequencies between POR and control groups.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Minor allele frequency</th>
<th>Allelic χ²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41308602</td>
<td>n=85</td>
<td>Control, n=85</td>
<td></td>
</tr>
<tr>
<td>rs138281369</td>
<td>0.046 (n=3)</td>
<td>0</td>
<td>4.003</td>
</tr>
<tr>
<td>rs104894767</td>
<td>0.015 (n=1)</td>
<td>0.011 (n=1)</td>
<td>0.036</td>
</tr>
<tr>
<td>rs104894763</td>
<td>0.015 (n=1)</td>
<td>1</td>
<td>0.316</td>
</tr>
<tr>
<td>rs3897937</td>
<td>0.615 (n=40)</td>
<td>0.459 (n=39)</td>
<td>3.621</td>
</tr>
<tr>
<td>rs796052131</td>
<td>0</td>
<td>0.035 (n=3)</td>
<td>2.340</td>
</tr>
<tr>
<td>rs796052132</td>
<td>0.061 (n=4)</td>
<td>0.023 (n=2)</td>
<td>1.385</td>
</tr>
</tbody>
</table>

The Table comprises the results of haplotype analysis. The associations were measured by odds ratio (OR) within 95% confidence interval.

Table 4: Combined frequencies of minor variant-comprising haplotypes in POR and control groups.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>POR, n=85</th>
<th>Control, n=85</th>
<th>OR, 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs41308602; rs138281369; rs104894767; rs3897937; rs796052131</td>
<td>0.767</td>
<td>0.550</td>
<td>2.7 (1.25; 5.96)</td>
</tr>
<tr>
<td>rs41308602; rs104894763; rs3897937</td>
<td>0.676</td>
<td>0.458</td>
<td>2.47 (1.20; 5.14)</td>
</tr>
</tbody>
</table>

The control group-specific novel c.607T variant gives no amino acid substitution (CTA, corresponding to leucine, is changed to synonymous TTA). Overall, the usage of these codons in human genome is equal (each of them accounts for 7% of the total number of leucines). However, specific tRNA for CTA is less abundant in the ovary than tRNA for TTA [24], and the c.607 C>T substitution may locally increase BMP15 mRNA translation rates thus influencing the protein production. The novel single nucleotide deletion c.-8 delC, more common in the POR group than in the controls, is located in the 5’ untranslated region of BMP15 mRNA in position 42. It does not interfere with the reading frame, but may affect initial steps of the translation.

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Conclusion

We report two novel polymorphic sites in BMP15 gene sequence, plausibly related to POR phenotypes. Examination of larger patient cohorts is required to further elucidate their connection with the phenomenon of POR in in vitro fertilization programs.

Acknowledgements

The study was supported by Ministry of Healthcare of the Russian Federation (grant #2012-02 reg. #01201256341). We acknowledge Prof Denis Rebrikov for participation in the writing and helpful discussions.

References


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