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ARTICLE

A single nucleotide polymorphism in BMP15 is associated with high response to ovarian stimulation

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Abstract There is substantial variability in ovarian response to exogenous gonadotrophins in women undergoing ovarian stimulation for IVF. Genetic variation in signalling pathways of the ovary may influence ovarian stimulation outcome. One previous study showed an association between single nucleotide polymorphisms (SNP) in the gene for bone morphogenetic protein 15 (BMP15) and ovarian hyperstimulation syndrome (OHSS). This article presents a retrospective case-controlled genetic-association study designed to test the association between SNP in the BMP15 gene and two clinically important outcomes of ovarian stimulation: low and high response. Blood samples from 53 high responders, 38 low responders and 100 controls were analysed for five SNP of interest. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated by a multivariate logistic regression model. We found an association between the BMP15 −9G allele and high response to ovarian stimulation (OR = 2.7, 95% CI = 1.3–5.7). This association confirms previous findings in a different population and strengthens the case for an association between this SNP and ovarian stimulation outcome.

KEYWORDS: BMP15, FSH, IVF, ovarian response, polymorphisms

Introduction

There is substantial variability in ovarian response to exogenous gonadotrophins in women undergoing ovarian stimulation for IVF. A low response reduces the likelihood of successful treatment (Klinkert et al., 2005; Saldeen et al., 2007). At the other end of the spectrum, patients with high response are at risk of developing ovarian hyperstimulation

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syndrome (OHSS) (Lee et al., 2008). Genetic variation could influence ovarian stimulation outcome. Most inter-individual genetic variation consists of single nucleotide polymorphisms (SNP). Several studies have investigated the possible associations between SNP and ovarian stimulation outcome. Much of this work was reviewed by Simoni et al. (2008), who concluded that SNP in the FSH receptor (FSHR) are associated to adverse outcomes of ovarian stimulation. SNP in genes encoding other molecules involved in follicular development have been investigated for association to stimulation outcome as well. Amongst these are the oestrogen receptors (Altmae et al., 2007), luteinizing hormone (Alviggi et al., 2009), the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) (Thaler et al., 2006) and anti-Müllerian hormone (AMH) and its receptor AMHR2 (Hanevik et al., 2010). Most of these studies are candidate gene-association studies that often need confirmation in other study populations. As far as is known, only one genome-wide association study on ovarian stimulation outcome has been published, with no statistically significant findings (van Disseldorp et al., 2009).

Another protein involved in follicular development is the bone morphogenetic protein 15 (BMP15). Moron et al. (2006) found a statistically significant association between high response to ovarian stimulation and SNP in the BMP15 gene. BMP15 is a member of the transforming growth factor β superfamily. In humans BMP15 is secreted from the oocyte of primary follicles (Aaltonen et al., 1999; Shimasaki et al., 2004). One recent study in human primordial follicles found BMP15 protein in oocytes and BMP15 mRNA transcripts in granulosa cells (Margulis et al., 2009). This opens the possibility of a role for BMP15 in the recruitment of primordial follicles as well. BMP15 acts by binding to BMPR-IA, BMPR-IB and BMPR-II (Moore et al., 2003). These receptors are present in human granulosa cells (Moore et al., 2003; Shimasaki et al., 2004), including those of primordial follicles (Abir et al., 2008). The BMP15 ligand—receptor complex activates SMAD proteins 1, 5 and 8 that influence gene expression in the nucleus of granulosa cells (Moore et al., 2003). BMP15 has a range of effects on the granulosa cells (Otsuka, 2010). Among these is a decrease in the expression of FSHR on the granulosa cell surface (Otsuka et al., 2001). A SNP in the gene for BMP15 that renders the protein less bioactive or inhibits its secretion would theoretically increase the follicles' sensitivity to FSH. An activating SNP could have the opposite effect. Di Pasquale et al. (2004) reported a case of two sisters where a substitution in the BMP15 pro-region caused hypergonadotrophic ovarian failure. The BMP15 gene is an interesting candidate for genetic-association studies in relation to ovarian stimulation. Thus, a study was designed to determine if SNP in the BMP15 gene are associated with two clinically important outcomes of ovarian stimulation: high and low response.

Materials and methods

Patients

A retrospective case-controlled genetic-association study was designed and approved by the regional medical ethics board. All patients undergoing ovarian stimulation from January 2003 to June 2009 at the Fertilitetsklinikken Sør, Porsgrunn, Norway were eligible to participate in the study. The criteria for inclusion/exclusion are presented in Table 1. Cases included in the study were patients who showed a high or low response to a standard dose of exogenous recombinant FSH (rFSH) or human menopausal gonadotrophin (HMG). Patients with a normal response to the same medication were used as controls. Patients with previous ovarian stimulation were accepted in the study. No specific effort was made to match cases to controls as this would have required a separate control group for each case group. The inclusion/exclusion criteria were set strictly to decrease heterogeneity between the case groups and the control group to an acceptable level.

The patients included in the study did at no point during the ovarian stimulation receive a daily dose of exogenous gonadotrophins >200 IU or <150 IU (<100 IU in the high-responder group). This criterion was set to minimize variation in stimulation protocols between groups which is known to influence stimulation outcome. To be considered as a high responder, a minimum of 15 oocytes had to be retrieved in accordance with earlier studies that identify patients with an excessive response to ovarian stimulation (Enskog et al., 1999; Popovic-Todorovic et al., 2003). High-responder patients also had to show signs of moderate or severe OHSS using the criteria set by Rizk and Aboulghar (1999). This criterion was set to decrease the heterogeneity of patients in the high-responder group and exclude patients for whom a high response to stimulation was less disadvantageous. For the low responders, in order to increase the chance of identifying patients with a genetic background for their low response to stimulation, the analysis excluded patients with conditions that could be associated with low response such as previous adnexal surgery, ovarian endometriosis and ovarian cysts. A total of 338 patients were asked to participate in the study, and the study received 191 blood samples and signed consent forms (100 from controls, 53 from high responders and 38 from low responders). The indications for fertility treatment for the 191 patients were: male factor ($n = 97$), unexplained infertility ($n = 47$), tubal factor ($n = 42$), endometriosis ($n = 4$) and uterine factor ($n = 1$). The ethnicity of the patients was determined by photographs and information in the patient records. The clinical and treatment characteristics of the three groups are presented in Table 2.

Ovarian-stimulation protocol

The patients were pituitary desensitized with a long protocol using the gonadotrophin-releasing hormone agonist Nafarelin (Synarela; Pfizer, New York, USA) 800 μg daily from day 20 in the previous menstruation cycle, reduced to 400 μg daily from the start of rFSH/HMG injections. Ovarian stimulation was with subcutaneous injections of either rFSH (Puregon; Schering-Plough, Oss, The Netherlands or Gonal-F; Merck-Serono, Geneva, Switzerland) or HMG (Menopur; Ferring, Saint-Prex, Switzerland). The standard starting dose of rFSH/HMG was 150 IU/day. Patients who were judged to be at risk for high or low response to ovarian stimulation had their starting dose adjusted, without any specific standard of adjustment, but always within the