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ARTICLE

Ovarian response is affected by a specific histidine-rich glycoprotein polymorphism: a preliminary study

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Abstract Genetic polymorphisms involved in angiogenesis, apoptosis and chemokine signalling are associated with varying ovarian response and oocyte quality. The protein, histidine-rich glycoprotein (HRG), is involved in these processes, but its effect on ovarian response in IVF has not been previously studied. A single nucleotide polymorphism (SNP) in the *HRG* gene (C633T) seems to affect pregnancy results in IVF. Women with the C/C genotype had higher pregnancy rates, C/T had moderate rates and none of those in the T/T group conceived. The aim of this study was to investigate if the *HRG* C633T SNP affects ovarian response. The *HRG* C633T SNP genotype of 67 women with unexplained infertility undergoing IVF was analysed and related to medical data. The T/T genotype obtained fewer oocytes, including mature oocytes, despite higher dosages of FSH administered. Additionally, the highest proportion of women who had exclusively poor-quality embryos was in the T/T group. No differences in demographic factors known to affect these parameters were found. The results suggest that the *HRG* C633T SNP influences ovarian response. Further studies of this SNP may increase knowledge about the biological processes involved in oocyte development and, furthermore, improve predicted ovarian response and fertilization.

KEYWORDS: embryo development, histidine-rich glycoprotein, in-vitro fertilization, oocyte, ovarian response, single nucleotide polymorphism

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Introduction

The ultimate goal of IVF is to have a successful live birth of a healthy child; however, several variables during treatment indicate a higher or lower chance of pregnancy (van Loendersloot et al., 2010). The sensitivity of the ovaries to FSH given during ovarian stimulation is individual and is affected by factors such as age, body mass index (BMI), smoking, basal FSH levels, anti-Müllerian hormone (AMH) levels and antral follicle count (AFC) (Broekmans et al., 2006; Holte et al., 2011). The emerging best predictors of ovarian response are AMH and AFC (La Marca and Sunkara, 2014; Nelson, 2013). The sensitivity and specificity of all of the above mentioned factors are low, however, with considerable inter-individual differences in response (Broekmans et al., 2006).

A patient's response to IVF medications is also dependent upon her genetic makeup, including different variations owing to single nucleotide polymorphisms (SNP). For example, genetic variations of genes coding for LH (Alviggi et al., 2009), the oestrogen receptor (Altmae et al., 2007) and the FSH receptor (Perez Mayorga et al., 2000) have been associated with FSH sensitivity and ovarian response in IVF patients. Hence, it has been suggested that genetic screening may, in the future, help clinicians optimize treatment strategies and improve predictions of ovarian response and IVF outcome (Alviggi et al., 2012).

We have previously shown that a genetic variant of the histidine-rich glycoprotein (*HRG*) gene seems to affect pregnancy success rate in IVF (Nordqvist et al., 2011). The SNP at position 633 in the *HRG* gene consists of a cytosine (C633) or thymine (633T). A cytosine (C) at this position will code for a proline in the HRG protein at amino acid position 204 (sometimes denoted as position 186 if the signal protein is not included). A thymine (T), however, will code for a serine instead. The serine form allows for a glycosylation in the protein. This SNP has also been assigned the National Center for Biotechnology Information (NCBI) reference SNP identification tag rs9898. Women with unexplained infertility who are homozygous for the *HRG* C633 SNP (C/C) (also denoted previously as Pro/Pro) seem to have higher than expected pregnancy rates, heterozygous women (C/T) (also denoted as Pro/Ser) moderate rates and women homozygous for the *HRG* 633T SNP (T/T) (also denoted as Ser/Ser) have lower than expected pregnancy rates (Nordqvist et al., 2011). Additionally, this SNP is associated with recurrent spontaneous abortion, with the T/T group having an increased risk (Lindgren et al., 2013).

Histidine-rich glycoprotein is a plasma glycoprotein involved in biological systems, such as fibrinolysis and coagulation, the immunological response system, apoptosis and angiogenesis (Poon et al., 2011). These systems are known to be involved in oocyte development and pregnancy (Haller-Kikkatalo et al., 2012; Van Blerkom et al., 1997). Although its exact biomolecular function is unclear, HRG seems to be an adaptor molecule owing to its unique molecular structure, which allows it to interact with a multitude of different ligands. It has previously been reported to interact with molecules such as heparin, fibrinogen, plasmin and plasminogen, thrombospondin, vascular endothelial growth factor (VEGF) and various members of the fibroblast growth factor (FGF) family (Jones et al., 2005; Wake et al., 2009). Many of these ligands are associated with infertility (Barroso et al., 1999; Chaves et al., 2012; Ebisch et al., 2008; Richards, 2005). Low pH (Borza, 2005) or interaction with Zn^{2+} affects ligand binding through conformational changes in the HRG molecule (Jones et al., 2004). A recent study, using a new purification method, suggests that some previously reported functions of HRG, such as removal of necrotic cells, may not be through interactions between HRG and various ligands but, instead, due to the presence, activity of co-purified molecules in the HRG-complex, or both (Patel et al., 2013). This study by Patel et al. (2013) is also the first to suggest a possible phosphorylation of HRG. Thus, HRG is an adapter molecule, bringing together different ligands, suggesting that it regulates several important biological systems, many of which are associated with infertility (Blank and Shoenfeld, 2008).

Since the *HRG* C633T SNP seems to affect pregnancy results in IVF, it may be hypothesized that this SNP could affect ovarian response. The aim of this study was to investigate if the *HRG* C633T SNP is associated with ovarian response and fertilization in IVF.

Material and methods

Women diagnosed with unexplained infertility were recruited at an IVF Clinic (Fertilitetscentrum Stockholm) between 1 March 2010 and 6 February 2012. This study was part of a larger study in which 155 women with diverse causes of infertility were included. Each woman contributed a blood sample only once. All women had previously undergone assessment for cause of infertility. Women were screened for anovulation, including polycystic ovarian syndrome (PCOS) according to Rotterdam criteria (Rotterdam Eshre/Asrm-Sponsored Pcos Consensus Workshop Group, 2004), thyroid dysfunction, tubal factors, male factors, endometriosis and uterus anomalies. Basal FSH levels or AMH levels were tested on all women. Basal FSH levels below 13 IU/L or AMH levels above 0.7 ng/ml were considered normal. Sperm counts below the World Health Organization guideline levels (Tocci and Lucchini, 2010) were classified as male infertility. When the above mentioned factors were absent, the infertility was classified as unexplained. On the basis of the strong association between the *HRG* C633T SNP and unexplained infertility, the analysis was continued focusing on this sub-group of 67 women. Four women had previously given birth to a child.

Treatment background

The *HRG* C633T SNP was successfully analysed in 67 women. The mean treatment number was 1 ± 1 SD (minimum = 1, maximum = 5). Starting dosages of FSH were determined individually based on anticipated ovarian response and, whenever possible, on previous treatment results. Ultrasound evaluation began on stimulation day 5–10 and repeated if necessary. Dosages could be changed at the ultrasound depending on results. A gonadotropin releasing hormone (GnRH) agonist protocol was used for 12 women composed of buserelin (Suprecur; Sanofi-Aventis, Stockholm, Sweden) (n = 3); nafarelin (Synarela; Pfizer, Sollentuna,