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ARTICLE

Association of a promoter polymorphism in *FSHR* with ovarian reserve and response to ovarian stimulation in women undergoing assisted reproductive treatment

Dalia Tohlob [a,b], Ekbal Abo Hashem [b], Nawal Ghareeb [b], Mohammed Ghanem [c], Reham Elfarahaty [b], Helen Byers [a], Philip Pemberton [d], Stephen A Roberts [e], William G Newman [a,1], Lamiya Mohiyiddeen [f,*,1]

[a] *Manchester Centre for Genomic Medicine, University of Manchester, Manchester, United Kingdom;* [b] *Clinical Pathology Department, Faculty of Medicine, Mansoura University Mansoura, Egypt;* [c] *Obstetrics and Gynaecology Department, Faculty of Medicine, Mansoura University Mansoura, Egypt;* [d] *Department of Clinical Biochemistry, Manchester Royal Infirmary, Manchester, United Kingdom;* [e] *Centre for Biostatistics, Institute of Population Health, Manchester Academic Health Sciences Centre (MAHSC), University of Manchester, Manchester, United Kingdom;* [f] *Department of Reproductive Medicine, Central Manchester University Hospitals NHS Foundation Trust, Manchester, United Kingdom*

* Corresponding author. *E-mail address:* Lamiya.Mohiyiddeen@cmft.nhs.uk (L Mohiyiddeen). [1] These authors contributed equally to the study.

Dr Dalia Tohlob graduated from medical school, Mansoura, Egypt in 2004, and has a Masters degree in clinical pathology. She is currently a PhD scholar at Manchester University, Manchester Centre of Genomic Medicine. She is working in the area of genetic polymorphisms and their association with ovarian response in females undergoing assisted reproduction.

Abstract Previous studies have suggested an association between a variant in the promoter region of the FSHR gene and diminished response to controlled ovarian hyperstimulation (COH) in women undergoing assisted reproduction. *FSHR* -29G>A was genotyped in 559 women undergoing their first cycle of COH for IVF/intracytoplasmic sperm injection (ICSI) using TaqMan allelic discrimination assay. Correlation and regression analysis was performed to assess the relationship between *FSHR* promoter genotypes and markers of ovarian reserve and measures of response to COH, including the number of oocytes retrieved, gonadotrophin dose used and the live-birth rate. There were no statistically significant differences between the genotype frequencies and the markers of ovarian reserve or the early measures of response to COH. However, the live-birth rate was higher for women carrying the variant A allele (odds ratio [OR] 1.37; 95% confidence interval [CI] 1.02–1.84 per allele). This relationship did not reach statistical significance after adjustment

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for the number of embryos transferred (OR 1.33; 95% CI 0.98–1.83 per allele). Results from this study do not provide evidence that the *FSHR* -29G>A variant can be used in the individualization of the treatment protocol for women undergoing IVF/ICSI.

KEYWORDS: FSHR, hyperstimulation, IVF, ovarian response, polymorphism

Introduction

The principal goal of controlled ovarian hyperstimulation (COH) is to harvest a high number of mature oocytes, which can be used for IVF (Boudjenah et al., 2012; Grady et al., 2012). Response to the drugs used in COH is variable and sometimes unexpected. Some women have a hyper-response which can be exacerbated resulting in ovarian hyperstimulation syndrome (OHSS), a life-threatening condition characterized by ovarian enlargement and abdominal fluid accumulation even with low gonadotrophin doses (Humaidan et al., 2010). In contrast, some women have a low response despite high doses of gonadotrophins and normal ovarian reserve. These situations lead to psychological and physical morbidity and have significant economic implications (Desai et al., 2013).

Individualization of treatment protocols is an attractive strategy to improve IVF outcomes. However, although many studies have been conducted to define predictors of response to COH, to date there is insufficient evidence of utility to adopt genetic biomarkers into clinical practice (Ferraretti et al., 2011).

Studies assessing biomarkers of response to COH have used different outcome measures, potentially contributing to differences in results. Predictors of inadequate ovarian response include advanced age (≥40 years) and low ovarian reserve parameters (Binder et al., 2012), including anti-Müllerian hormone (AMH) concentrations and the antral follicle count (AFC). These measures of ovarian reserve have been used to individualize the dose of gonadotrophin although they do not always improve the outcome of assisted reproduction (Trevisan et al., 2014).

Genetic variability among individuals has been studied to determine the effect on the outcome of COH, including variations in the AMH, AMH receptor, luteinizing hormone (LH), LH receptor, oestrogen receptors and folate metabolizing genes (Altmae et al., 2011). However, the most extensively studied is the *FSHR* gene, encoding the follicle stimulating hormone receptor. A number of single nucleotide polymorphisms (SNP) in *FSHR*, have been associated with measures of ovarian response (Simoni et al., 2002; Wunsch et al., 2005). Two common variants in *FSHR*, c.919G>A, p.(Thr307Ala) and c.2039A>G, p.(Asn680Ser) have been extensively studied. In the cohort described here, there was no significant association between these variants and either ovarian response or reserve parameters (Mohiyiddeen et al., 2012, 2013).

A variant in the 5′ untranslated region of *FSHR*, -29G>A (rs1394205), has been associated with changes in the receptor expression due to changes in transcriptional factor binding sites (Nakayama et al., 2006; Wunsch et al., 2005). However, no association between *FSHR* -29G>A and basal FSH or oestradiol concentrations was established in 202 females undergoing IVF treatment (Wunsch et al., 2005). In contrast, a small study of 50 Indian women reported an association between the homozygous variant genotype (AA) with decreased preovulatory follicle count, the number of oocytes retrieved and lower pregnancy rates (Achrekar et al., 2009). In a further study of 100 Indian women, homozygosity for the variant was also associated with inadequate ovarian response (Desai et al., 2011). A Turkish study of 102 infertile females found no relation between this variant and baseline FSH concentrations (Ilgaz et al., 2015).

Clinical equipoise exists as to whether the *FSHR* promoter gene variant provides useful information to indicate ovarian reserve or the outcome of IVF treatment. We therefore assessed the relationship between the -29G>A *FSHR* variant and the ovarian reserve parameters (FSH, AMH, AFC); the primary outcome measure of the number of eggs retrieved and secondary outcome measures of total gonadotrophin dose used and live-birth event in a previously collected and phenotyped cohort of 603 women undergoing assisted reproduction.

Materials and methods

Consecutive women attending a tertiary referral centre for reproductive medicine in Manchester, UK were recruited between March 2009 and August 2010.

Inclusion criteria are as follows: (i) age <40 years; (ii) body mass index (BMI) >19 and <30 to meet eligibility criteria for government funded IVF treatment in Greater Manchester, UK; (iii) first cycle of IVF treatment; (iv) presence of two ovaries and no previous ovarian surgery or radiation therapy; and (v) no hormonal therapy was used in the six months prior to recruitment.

A total of 603 women were recruited. Of these, 19 women did not proceed with treatment. No blood sample for genotyping was available for 25 women and the analysis was confined to the remaining 559.

421 women from this study were included in a previous report of different *FSHR* variants (Mohiyiddeen et al., 2013) and 239 women were included in a study assessing the relationship between a *BMP15* variant and ovarian response and reserve parameters (Cerra et al., 2014). The protocol was approved in 2008 by the South Manchester Research Ethics Committee (REC ref no. 08/H1003/212). Written informed consent was obtained from all participants. As this study utilised a pre-existing cohort with a fixed sample size and unknown genotype frequencies, power calculations were inappropriate.

Basic hormonal assessment

Blood samples were taken on day 3 of spontaneous menstrual cycle or after withdrawal bleeding in women with anovulatory cycles for measurement of serum FSH, serum AMH