

# Clinical impact of scavenger receptor class B type I gene polymorphisms on human female fertility

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**BACKGROUND:** The goal of this study was to evaluate the association of *SCARB1* single nucleotide polymorphisms (SNPs) and fertility outcomes in women undergoing IVF.

**METHODS:** Between November 2007 and March 2010, granulosa cells and follicular fluid were collected from women undergoing IVF. Five *SCARB1* SNPs were sequenced and progesterone levels were measured in the follicular fluid. Fertility measurements were defined as the presence of gestational sac(s) and fetal heartbeat(s).

**RESULTS:** The study group consisted of 274 women (mean age of  $36.4 \pm 4.6$  years). The racial/ethnic composition was 55% Caucasian ( $n = 152$ ), 25% African-American ( $n = 68$ ), 12% Asian ( $n = 34$ ), 5% Hispanic, ( $n = 14$ ) and 2% other ( $n = 6$ ). There was a significant difference in the genotype frequencies of the *SCARB1* SNPs across the groups. Subjects who were homozygous for the minor allele in the rs5888 SNP had higher follicular progesterone levels than those who were homozygous for the major allele ( $P = 0.03$ ). In the Caucasian group, carriers of the minor A allele of the rs4238001 SNP had lower follicular progesterone levels compared with homozygous carriers of the major G allele ( $P = 0.04$ ). In this group, follicular progesterone levels were highly predictive of the rs4238001 SNP ( $P = 0.03$ ). In the entire cohort, minor allele carriers of rs4238001 did not have any viable fetuses at Day 42 following embryo transfers ( $P = 0.04$ ). In the African-American group in particular, there was also an association between rs10846744 and gestational sac(s) ( $P = 0.006$ ), and fetal heartbeat(s) ( $P = 0.005$ ).

**CONCLUSIONS:** In part, *SCARB1* rs4238001 and rs10846744 SNPs may contribute to human female infertility.

**Key words:** lipoprotein receptors / fertility / progesterone / genotypes

## Introduction

Scavenger receptor class B type I (SR-BI) is a physiologically relevant lipoprotein receptor that functions by mediating the selective uptake of neutral lipids in highly expressive tissues such as the liver, adrenals, and ovaries (Acton *et al.*, 1996; Landschulz *et al.*, 1996; Rigotti *et al.*, 1997). It is thought to exert a major role in reverse cholesterol transport, a process by which plasma cholesterol is delivered to the liver for bile acid production (Fielding and Fielding, 1995). In addition, SR-BI has been shown to mediate cholesterol uptake in steroidogenic tissue for hormone production (Reaven *et al.*, 1995; Azhar *et al.*, 1998a,b, 1999; Swarnakar *et al.*, 1999). In humans, the SR-BI gene (*SCARB1*) (formerly referred to as CLA-1) is localized to chromosome 12 (12q24.31) and contains 12 exons (Calvo and Vega, 1993).

In female mice, complete deficiency of SR-BI has been associated with reduced fertility, with these mice demonstrating abnormal preimplantation embryo development (Trigatti *et al.*, 1999). More recently, Jimenez *et al.* (2010) reported that SR-BI knockout (KO) mice had 50% lower serum progesterone levels when compared with wild-type mice. Far less is known regarding the role of SR-BI in human female infertility. We had previously shown that infertile women with low SR-BI RNA levels isolated from granulosa cells following hCG-stimulated oocyte retrievals had ~50% lower levels of plasma estradiol levels, and a significantly lower number of retrieved and fertilized oocytes (Velasco *et al.*, 2006). Using an *in vitro* approach, we recently reported that deficiency of SR-BI protein was significantly associated with markedly lower progesterone secretion in human granulosa cells (Kolmakova *et al.*, 2010). The effect of SR-BI deficiency

on progesterone secretion was independent of lipoproteins in the culture media, and was significantly associated with down-regulation of key steroidogenic genes such as steroidogenic acute regulatory protein (StAR), P450 side-chain cleavage (P450scc) and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD).

More is known regarding the role of SR-BI in human lipid metabolism and atherosclerosis risk. A number of epidemiological studies have shown significant associations between *SCARB1* single nucleotide polymorphisms (SNPs), lipids, BMI and glucose levels (Acton *et al.*, 1999; McCarthy *et al.*, 2003a,b, 2009; Rodriguez-Esparragon *et al.*, 2005; Roberts *et al.*, 2007; West *et al.*, 2009; Naj *et al.*, 2010). For instance, Acton *et al.* (1999) showed that the synonymous SNP, rs5888, was significantly associated with low-density lipoprotein cholesterol levels, while we previously showed that this SNP was associated with higher high-density lipoprotein (HDL) cholesterol levels in younger women participants of the Amish Family Diabetes Study (Roberts *et al.*, 2007). More recently, we showed that *SCARB1* SNPs were significantly associated with increased subclinical atherosclerosis in participants of the Multi-Ethnic Study of Atherosclerosis (Naj *et al.*, 2010). We also determined the functional effect of the rs4238001 SNP (a non-synonymous SNP that causes an amino acid change from glycine to serine at Position 2 of the SR-BI protein) on SR-BI protein expression in human macrophages (West *et al.*, 2009).

The objective of this current study was to examine the association of these key *SCARB1* SNPs with fertility measurements in women undergoing controlled-ovarian stimulation and IVF. The rationale for studying these infertile women is the availability of granulosa cells, which express SR-BI and can be collected at the time of oocyte retrievals. Overall, our results showed that the non-synonymous *SCARB1* SNP, rs4238001, was significantly associated with lower follicular progesterone levels, especially in the Caucasian group. Importantly, in the entire group, rs4238001 was significantly associated with poor fetal viability.

## Materials and Methods

### Study demographics

In a prospective study design, between November 2007 and March 2010, granulosa cells were isolated from 320 women undergoing controlled-ovarian hyperstimulation (COH) and IVF at The Johns Hopkins Fertility Center. The study design was previously reported (Velasco *et al.*, 2006). We did not have access to lipid profiles or serum progesterone levels as these were not routinely ascertained for each subject prior to initiation of the IVF protocol. There were 46 subjects removed from the final analysis because 19 were normal healthy oocyte donors, and the remaining 29 subjects had multiple IVF treatments. Subjects provided informed written consent for the IVF treatment and use of biological samples for genetic testing. The study was approved by the Johns Hopkins Institutional Review Board.

### Materials

Chemical reagents and solvents were obtained from Sigma-Aldrich (St Louis, MO, USA), the MiniKit QIAmpDNA was from Qiagen (Valencia, CA, USA) and the progesterone ELISA kit was from ALPCO (Salem, NH, USA).

### Granulosa cell retrieval and isolation

Follicular aspirates from each subject were centrifuged at 1500g for 10 min at 4°C (Velasco *et al.*, 2006). Follicular fluid was then collected and an aliquot was extracted for progesterone measurement. The cell pellet was re-suspended in phosphate-buffered saline (PBS), overlaid onto 40% (v/v) Percoll solution and centrifuged at 2500g at 4°C. Granulosa cells at the Percoll–PBS interface were aspirated, re-suspended in PBS and pelleted by centrifugation at 1500g. This step was repeated two times and the recovered cells were processed for genomic DNA extraction.

### Clinical fertility measurements

Subjects underwent COH and oocyte aspiration as previously described (Velasco *et al.*, 2006). Embryo transfers were performed on Day 3 or 5 after retrieval. I.M. progesterone (50 mg daily) or vaginal progesterone (100 mg three times daily) were initiated the day following oocyte retrieval for luteal phase support. A serum pregnancy test was performed 14 days after embryo transfer by measuring serum hCG. Clinical pregnancy was defined as the presence of a gestational sac(s) and the data were coded as categorical '0 = no' for no gestational sac(s) and '1 = yes' for the presence of gestational sac(s). Patients were followed by transvaginal ultrasound until the detection of fetal heart motion (Day 42 post-embryo transfer) and the data were coded as categorical '0 = no' for heartbeat(s) and '1 = yes' for heartbeat(s).

### Follicular fluid analyses

Progesterone levels were measured in follicular fluid extracts. The rationale for measurement of progesterone in follicular fluid was based on its availability and the direct contribution of ovarian progesterone secretion in the follicular fluid. Each 100  $\mu$ l sample of follicular fluid was placed into a glass tube and 1 ml of petroleum ether was added (Plaino *et al.*, 2000). The tube was subjected to vortexing for 30 s at maximum speed to separate the organic and inorganic phases. The organic phase was transferred into a new glass tube and the solvent was evaporated under a stream of N<sub>2</sub>. The residue was dissolved in PBS and analyzed by ELISA using a commercially available kit. The intra- and inter-assay coefficient of variation for the assay is 7.3 and 11.3%, respectively.

### DNA sequencing

Genomic DNA was extracted from granulosa cells using a QIAamp DNA Mini Kit. The following *SCARB1* SNPs (gene location) were characterized by direct sequencing in both directions of PCR products as previously described (West *et al.*, 2009): rs4238001 (exon 1), rs10846744 (intron 1), rs5891 (exon 3), rs2278986 (intron 3) and rs5888 (exon 8). Sequence comparisons were determined using the Sequencher Program v.4.0 (Gene Code). The primer sequences and PCR conditions are available from the authors upon request. The rationale for selecting these particular SNPs was based on previously reported significant associations of these SNPs with phenotypic traits (Acton *et al.*, 1999; McCarthy *et al.*, 2003a,b, 2009; Rodriguez-Esparragon *et al.*, 2005; Roberts *et al.*, 2007; West *et al.*, 2009; Naj *et al.*, 2010).

### Statistical analysis

The differences in *SCARB1* genotype frequencies across the racial/ethnic groups and the association of *SCARB1* SNPs with clinical fertility measurements were measured by  $\chi^2$  analysis. The association of *SCARB1* SNPs with follicular progesterone levels was performed using one-way analysis of variance (ANOVA). Each *SCARB1* SNP was added to the multivariate stepwise regression analyses individually and together to assess whether the SNPs were independent predictors of follicular progesterone levels. Threshold significance values for selection and retention in the stepwise

analysis were 0.25 and 0.10, respectively. All analyses were performed using JMP Genomics 4.2 (SAS Institute, Cary, NC, USA). Probability values  $<0.05$  were considered statistically significant.

## Results

The study population consisted of 274 adult women with a mean age of  $36.4 \pm 4.6$  years (range 23–46 years). The self-described racial/ethnic composition of the group consisted of 55% Caucasian ( $n = 152$ ), 25% African-American ( $n = 68$ ), 12% Asian ( $n = 34$ ), 5% Hispanic, ( $n = 14$ ) and 2% other ( $n = 6$ ). Other general characteristics of the entire group included a mean BMI of  $26.8 \pm 7.1$  (range 16.8–53.3), and previous IVF attempts of  $1.8 \pm 1.5$ .

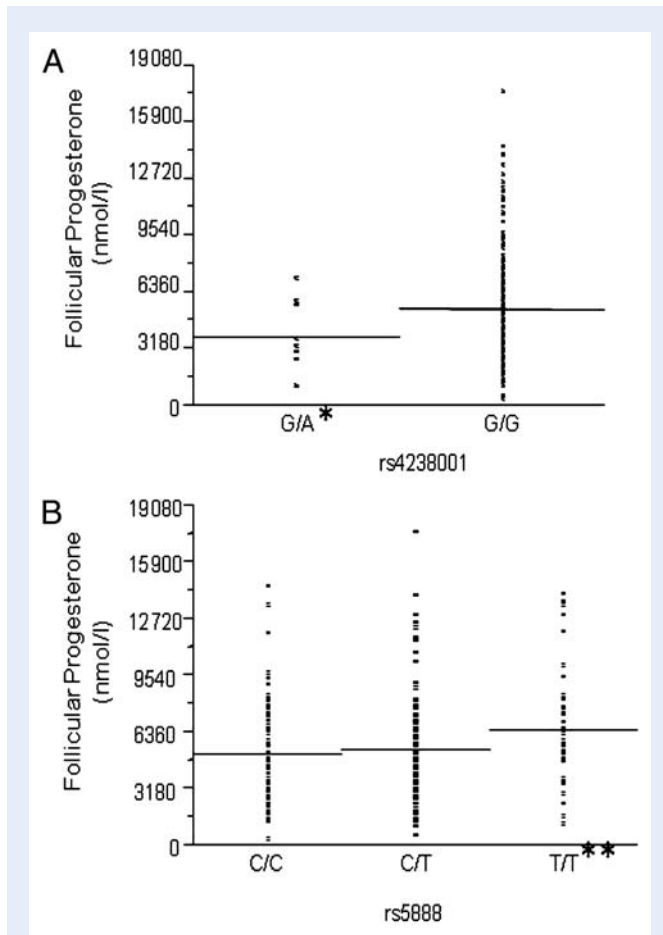
The gene location and genotype frequencies of the *SCARB1* SNPs in this population are shown in Table I. We observed significant differences between genotype frequencies of the rs10847644 ( $P < 0.0001$ ) and rs2278986 ( $P = 0.03$ ) across all the racial/ethnic groups. However, we did not observe differences in the *SCARB1* genotype frequencies between the Caucasian and Hispanic groups, nor between the African-American and Asian groups. We did observe significant differences in the rs10846744 ( $P < 0.0001$ ), rs2278986 ( $P = 0.01$ ) and rs5888 ( $P = 0.01$ ) genotypes between the Caucasian and African-American groups. The genotype frequency of rs10847644 was also significantly different between Caucasians and Asians ( $P < 0.0001$ ).

We had previously shown that silencing SR-BI protein expression in immortalized human granulosa cells was associated with significantly lower progesterone secretion (Kolmakova et al., 2010); therefore, we next examined the univariate association of the *SCARB1* SNPs with follicular progesterone levels. As shown in Fig. 1A, for the entire group, carriers of the minor A allele for the rs4238001 SNP had lower follicular progesterone levels compared with homozygous carriers of the major G allele (29% lower,  $P < 0.08$ ). In contrast, we found that subjects who were homozygous for the minor T allele of the rs5888 SNP had significantly higher follicular progesterone levels compared with subjects homozygous for the major C allele (homozygous major CC:  $5061 \pm 285$  nmol/l; heterozygous CT:  $5367 \pm 260$ ; homozygous minor TT:  $6498 \pm 462$ ) ( $P = 0.03$ ) (Fig. 1B). In the Caucasian group, carriers of the minor allele for rs4238001 had lower follicular progesterone levels ( $2528 \pm 1517$ ) when compared with homozygous carriers of the major allele ( $5629 \pm 254$ , 55% lower,  $P = 0.04$ ) (Fig. 2A). In the Caucasian group, homozygous carriers of the minor allele for rs5888 had higher follicular progesterone levels (homozygous major CC:  $5148 \pm 450$  nmol/l; heterozygous CT:  $5346 \pm 359$ ; homozygous minor TT:  $7041 \pm 623$ ) ( $P = 0.04$ ) (Fig. 2B). In the African-American group, there were no significant univariate associations of *SCARB1* SNPs with follicular progesterone levels. The population size for Hispanics and Asian-Americans was too small for further subanalysis, and these groups were not analyzed further.

We also performed multivariate regression analysis in the Caucasian group using age, BMI, baseline FSH levels, and baseline LH levels, then in a stepwise fashion separately included each of the five *SCARB1* SNPs as independent covariates in the initial model, with follicular progesterone as the dependent variable. Following stepwise regression, only the rs4238001 SNP remained as an independent predictor of follicular progesterone levels ( $P = 0.03$ ). None of the other variables, including BMI, were independent predictors of follicular progesterone levels.

**Table I** Gene location and genotype frequencies of *SCARB1* SNPs.

Identification	Location	Amino acid change	Genotype frequency (%)				P-values across all groups		
			Caucasian	Hispanic	African-American	Asian-American			
rs4238001, G → A	Exon 1	Yes	GG (97)	GA (3)	AA (0)	AA (0)	AA (0)	n.s.	
rs10847644, C → G	Intron 1	No	CC (89)	CG (4)	GG (7)	GA (7)	GG (97)	GA (3)	
rs5891, G → A	Exon 3	Yes	GG (98)	GA (1)	AA (1)	GA (1)	GG (100)	CG (21)	GG (26)
rs2278986, T → C	Intron 3	No	TT (39)	TC (50)	CC (11)	TT (61)	TT (53)	TC (29)	CC (18)
rs5888, C → T	Exon 8	No	CC (39)	CT (51)	TT (17)	CC (29)	CT (37)	CT (50)	TT (12)

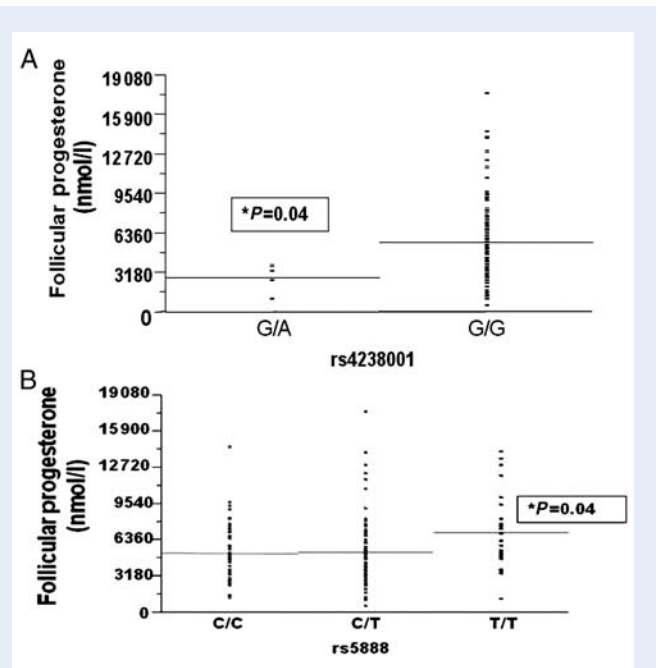


**Figure 1** Association of *SCARB1* SNPs with follicular progesterone levels in the entire cohort of infertile women undergoing IVF. *SCARB1* SNPs were genotyped by direct sequencing and follicular progesterone levels were measured in lipid extracts of follicular fluid using a commercially available assay. (A) \* $P < 0.08$  when compared with homozygous major G alleles by ANOVA analysis; (B) \*\* $P = 0.03$  compared with homozygous major C alleles.

Given that the rs4238001 remained as an independent predictor for follicular progesterone levels in the Caucasian group, we next examined the sensitivity and specificity of follicular progesterone as a predictor of the rs4238001 SNP using the receiver operating characteristic (ROC) analysis. As shown in Fig. 3, follicular progesterone was highly predictive with a sensitivity of 0.80 and a false-positive rate of 0.22 ( $P = 0.03$ ), with a cutoff value of  $<3682$  nmol/l (1158 ng/ml).

We next examined the association of each SNP with clinical fertility measurements, such as the number of retrieved and fertilized oocytes, the number of embryos transferred, clinical pregnancy and fetal heartbeat(s). Of these measurements, for the entire cohort, we found a significant association between rs4238001 and heartbeat(s), with carriers of the minor A allele ( $n = 9$ ) not having any viable fetuses at Day 42 post-embryo transfer (zero heartbeats) when compared with carriers homozygous for the major G allele ( $n = 63$  with heartbeats,  $P = 0.04$ ,  $\chi^2$ ).

A significant association was also observed between rs10846744 and the number of retrieved oocytes (homozygous major



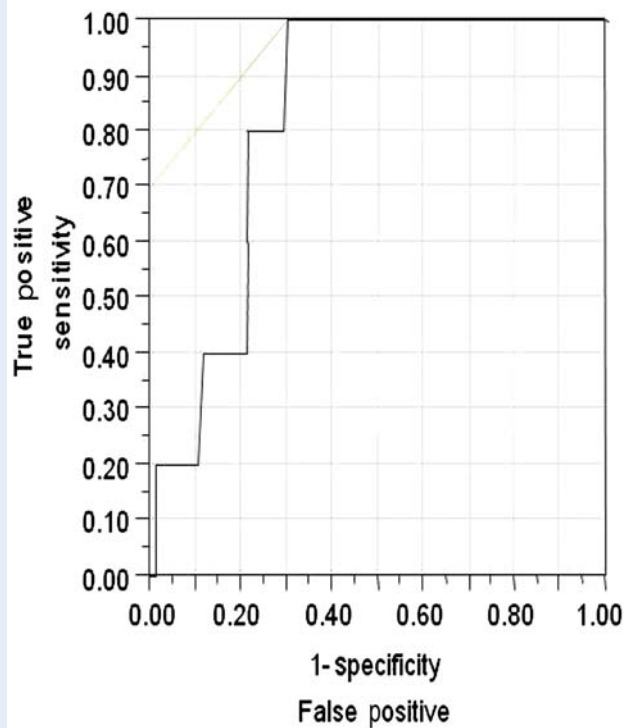
**Figure 2** Association of *SCARB1* SNPs with follicular progesterone levels in the Caucasian group. (A) \* $P = 0.04$  when compared with homozygous major GG alleles for the rs4238001 SNP by ANOVA analysis. (B) \* $P = 0.04$  when compared with homozygous major CC alleles for the rs5888 SNP by ANOVA analysis.

CC:  $9.3 \pm 0.5$ ; heterozygous CG:  $10.4 \pm 1.7$ ; homozygous minor GG:  $12.3 \pm 1.1$ ,  $P = 0.05$ ). For clinical pregnancy [categorical classification based on the presence (1) or absence (0) of gestational sacs], there was a significant association across the genotypes ( $P = 0.04$ ,  $\chi^2$ ): homozygous major CC ( $n = 158$ ): 28% with gestational sacs; heterozygous CG carriers ( $n = 15$ ): 60% with gestational sacs; and homozygous minor GG carriers ( $n = 38$ ): 32% with gestational sacs. For fetal heartbeats (categorical classification), there was a significant association across the genotypes ( $P = 0.03$ ,  $\chi^2$ ): homozygous major CC: 27% with heartbeats; heterozygous CG carriers: 60% with heartbeats; and homozygous minor GG carriers: 29% with heartbeats.

In the Caucasian group, we did not find a significant association between any of the *SCARB1* SNPs and the clinical fertility parameters. However, in the African-American group, we found a significant association between rs10846744 and clinical pregnancy ( $P = 0.006$ ,  $\chi^2$ ): homozygous major CC ( $n = 23$ ): 35% with gestational sacs; heterozygous CG carriers ( $n = 7$ ): 86% with gestational sacs; and homozygous minor GG carriers ( $n = 21$ ): 19% with gestational sacs. For fetal heartbeats ( $P = 0.005$ ,  $\chi^2$ ): homozygous major CC: 30% with heartbeats; heterozygous CG carriers: 86% with heartbeats; and homozygous minor GG carriers: 19% with heartbeats.

## Discussion

The goal of this study was to determine the association of certain key *SCARB1* SNPs with fertility measurements such as progesterone levels and measurements of embryo/fetal viability. Although there is a fair amount of knowledge regarding the role of SR-BI in rodent cardiovascular and reproductive physiology (Acton *et al.*, 1996; Rigotti *et al.*,



**Figure 3** ROC curve for prediction of rs4238001 based on follicular progesterone levels in the Caucasian group. Sensitivity 0.80, false-positive rate 0.22,  $P = 0.03$ .

2003), little is known regarding the role of *SCARB1* in human female reproductive physiology. We selected the *SCARB1* SNPs based on our previous work (Roberts et al., 2007; West et al., 2009; Naj et al., 2010) and that of others (Acton et al., 1999; McCarthy et al., 2003a,b, 2009; Rodriguez-Esparragon et al., 2005) showing significant associations of these SNPs with cholesterol levels and subclinical atherosclerosis. The rationale for studying the association of *SCARB1* SNPs with clinical fertility measurements in women undergoing IVF was due to the availability of granulosa cells and follicular fluid as by-products of oocyte retrievals.

Our results showing significantly lower follicular progesterone levels in women carriers of the minor A allele for rs4238001, especially in the Caucasian group (Fig. 2), are consistent with those recently reported by Jimenez et al. (2010) in the SR-BI KO female mice. These investigators reported that serum progesterone levels were 50% lower in the female SR-BI KO mice when compared with wild-type mice. We recently identified a mechanism by which SR-BI protein deficiency would impair progesterone secretion in cultured human granulosa cells (Kolmakova et al., 2010). We reported a novel, lipoprotein independent role of SR-BI deficiency in impairing *de novo* cholesterol synthesis, which led to down-regulation of key steroidogenic enzymes such as P450scc and  $3\beta$ -HSD.

In the univariate association analyses, we found that the rs5888 SNP was significantly associated with higher follicular progesterone levels in the entire group, and this was driven primarily by the association found in the Caucasian group (Fig. 2A and B). However, in the multivariate regression analyses, only the rs4238001 SNP remained as an

independent predictor of follicular progesterone levels. We found that the rs4238001 SNP was significantly associated with lower follicular progesterone levels, and follicular progesterone levels were in turn highly sensitive and specific in predicting the presence of the SNP (the ROC analysis, Fig. 3). This polymorphism is a non-synonymous SNP that causes an amino acid change (glycine  $\rightarrow$  serine) at Position 2 in the SR-BI protein. We have previously shown that subjects with hyperalphalipoproteinemia (HALP, defined as having HDL cholesterol  $> 60$  mg/dl) and carriers of the minor A allele had 50% lower SR-BI protein levels when compared with homozygous carriers of the major G allele (West et al., 2009). The minor allele frequency (MAF) of this SNP in this HALP population was 12%, and in other populations as reported in dbSNP ([http://www.ncbi.nlm.nih.gov/projects/SNP/snp\\_ref.cgi?rs=4238001](http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4238001)), the frequency varies between 2 and 13%. To the best of our knowledge, we are not aware of any studies linking HALP to human female infertility. Using an *in vitro* approach, we demonstrated that the rs4238001 SNP significantly increased SR-BI protein degradation; thus, this SNP is causal in inducing lower SR-BI protein expression (West et al., 2009). Therefore, our results in this current study of infertile women undergoing IVF showing an association of rs4238001 with lower follicular progesterone levels are consistent with our *in vitro* results of low SR-BI protein and low progesterone secretion, as well as the findings of significantly lower serum progesterone levels in SR-BI KO female mice. The ROC curve showed that follicular progesterone levels [values  $< 3682$  nmol/l (1158 ng/ml)] are highly predictive of SR-BI protein deficiency, strongly suggestive of the clinical utility of screening infertile women for SR-BI deficiency by genotyping for this particular *SCARB1* SNP.

SR-BI KO female mice are virtually infertile, with litter sizes being extremely small if spontaneous fertility occurs at all (A.R., personal observation; Trigatti et al., 1999; Miettinen et al., 2001). Trigatti et al. (1999) have characterized the SR-BI KO female mice as producing defective preimplantation embryos. Although we did not observe significant associations of *SCARB1* SNPs with qualitative measurements of embryo viability (blastocyst number and grade) (data not shown), we did find significant association of rs4238001 and rs10846744 with quantitative fertility measurements. The association of rs4238001 with heartbeats was particularly compelling as carriers of the minor A allele had no viable fetuses at Day 42 following embryo transfer; this despite routine pharmacological progesterone supplementation to all subjects following embryo transfer. In rodents, it has been observed that expression of P450scc and  $3\beta$ -HSD are significantly increased in endometrial glands at the time of implantation and this is associated with local progesterone production (Arensburg et al., 1999). SR-BI has also been shown to be expressed in human endometrial tissue and murine trophoblast giant cells (Ramachandran et al., 2001; Watanabe et al., 2010). In human granulosa cells, we have previously reported that silencing of SR-BI protein is associated with significantly reduced RNA expression of StAR, P450scc and  $3\beta$ -HSD (Kolmakova et al., 2010). Moreover, Piccinni et al. (1998) have shown the importance of progesterone in acting as an immunosuppressant by activation of TH2 helper cells. Thus while normal human endometrium has been shown to have low RNA expression levels of StAR, SCC and  $3\beta$ HSD RNA, this tissue produces very little progesterone following stimulation (Attar et al., 2009). It is still possible that reduced progesterone production in the pre-ovulatory follicular fluid in these patients influences both the oocyte

development and the quality of the endometrium prior to implantation. Certainly, iatrogenic progesterone is provided to these patients after oocyte retrieval, but the effects on the endometrium may have already occurred. Additionally, SR-BI has been shown to affect angiogenesis (Seetharam *et al.*, 2006) and nitric oxide (NO) production (Li *et al.*, 2002), and via these pathways, might have an effect on embryo implantation. It has been shown that progesterone can influence NO secretion in cultured human endometrial cells (Khorram and Han, 2009). Our results suggest that simple replacement of progesterone does not overcome problems with clinical fertility outcomes in infertile women carriers of the *SCARB1* rs4238001 and rs10846766 SNPs.

We had previously shown that rs10846744 was significantly associated with subclinical atherosclerosis in participants of the Multi-Ethnic Study of Atherosclerosis (Naj *et al.*, 2010). In this multi-ethnic population, we did observe differences in genotype frequencies of *SCARB1* SNPs, but did not observe racial/ethnic differences in association of these SNPs with phenotypic traits. In the MESA population, the MAF of this SNP varied across the racial/ethnic groups by 18–40%. The rs10846744 SNP is located in intron 1 and is not located at a traditional alternative splice site. To the best of our knowledge, neither is it a known seed or target for microRNA transcriptional regulation. It is possible that this SNP marks a region within the *SCARB1* locus that harbors a causal mutation; however, more work needs to be done to dissect the mechanism by which this intronic SNP affects SR-BI protein and function. In addition, based on our results in the MESA study, we did not find that rs10846766 was in linkage disequilibrium with other common *SCARB1* SNPs, nor do we have evidence, just yet, that this SNP directly affects transcription or translation of SR-BI. The underlying etiology for higher clinical pregnancy and fetal heartbeats in the heterozygous carriers is unclear, but does suggest a gain of function with this variant allele. Since this variant was not an independent predictor of follicular progesterone levels, and yet was associated with clinical fertility outcomes, this again suggests that the role of SR-BI on fertility outcomes is not directly mediated by progesterone levels, but likely through other SR-BI dependent pathways.

There were limitations to our study that merit further discussion. The selection of this study group was based on the availability of granulosa cells and follicular fluid by which to genotype key *SCARB1* SNPs and measure follicular progesterone levels, respectively. It is standard procedure for women undergoing IVF to receive pharmacological supplementation of progesterone following oocyte retrievals. Despite receiving progesterone supplementation (either by i.m. injection or vaginal suppositories), women carriers of the rs4238001 SNP were not able to maintain a viable pregnancy. This finding suggested that these modes of progesterone supplementation do not overcome the impaired fertility in women carriers of this SNP. At this time, we do not have a clear etiology for this observation, as we are limited in our ability to directly study human embryos and we did not have access to human endometrial tissue. It is possible that restoration of human fetal viability might require higher endometrial concentrations of progesterone (which in turn might affect factors such as local NO levels mediated through SR-BI), or possibly require alteration in the oxidant status or lipoprotein composition as observed in the restoration of fertility in SR-BI KO female mice treated with the antioxidant, cholesterol lowering medication, probucol (Miettinen *et al.*, 2001). The hypothesis that lipoprotein metabolism might have a beneficial effect on fertility was

also suggested by studies reported by Yesilaltay *et al.* (2006), in which these investigators showed that restoration of hepatic SR-BI expression by adenoviral transfection in SR-BI KO female mice normalized the lipoprotein profile and restored fertility. The importance of maternal lipoproteins on embryo viability has also been previously reported (McConihay *et al.*, 2000; Woollett, 2008).

In conclusion, while there is considerable knowledge regarding the role of SR-BI in rodent reproductive physiology, there is far less known about SR-BI in human female fertility. We have now shown that SR-BI exerts an independent effect on follicular progesterone levels, and follicular progesterone levels can be highly sensitive and specific predictors of rs4238001. This SNP and rs10846744 were also significantly associated with poor fetal viability, which may indicate a significant underlying mechanism for SR-BI in human reproduction which is highlighted even in the setting of COH, IVF and exogenous hormone supplementation. Since we are the first to make this observation, it is important that other groups replicate our findings. Overall, the results from our study suggest an important role of SR-BI in human female reproductive physiology.

## Authors' roles

Dr Yates collected clinical data, performed data analyses, and participated in the drafting of the manuscript; Dr. Kolmakova participated in the study design and data collection; Dr. Zhao collected data samples and participated in the study design; and Dr. Rodriguez designed the study, collected samples, performed data analysis, and drafted the original manuscript.

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## References

- Acton S, Rigotti A, Landschulz KT, Xu S, Hobbs HH, Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science* 1996;**271**:518–520.
- Acton S, Osgood D, Donoghue M, Corella D, Pocovi M, Cenarro A, Mozas P, Keilty J, Squazzo S, Woolf E *et al.* Association of polymorphisms at the SR-BI gene locus with plasma lipid levels and body mass index in a white population. *Arterioscler Thromb Vasc Biol* 1999;**19**:1734–1743.
- Arensburg J, Payne AH, Orly J. Expression of steroidogenic genes in maternal and extraembryonic cells during early pregnancy in mice. *Endocrinology* 1999;**140**:5220–5232.
- Attar E, Tokunaga H, Imir G, Yilmaz MB, Redwine D, Putman M, Gurates B, Attar R, Yaegashi N, Hales DB *et al.* Prostaglandin E2 via steroidogenic factor-1 coordinately regulates transcription of steroidogenic genes necessary for estrogen synthesis in endometriosis. *J Clin Endocrinol Metab* 2009;**94**:623–631.

- Azhar S, Nomoto A, Leers-Sucheta S, Reaven E. Simultaneous induction of an HDL receptor protein (SR-BI) and the selective uptake of HDL-cholesteryl esters in a physiologically relevant steroidogenic cell model. *J Lipid Res* 1998a;**39**:1616–1628.
- Azhar S, Tsai L, Medicherla S, Chandrasekher Y, Giudice L, Reaven E. Human granulosa cells use high density lipoprotein cholesterol for steroidogenesis. *J Clin Endocrinol Metab* 1998b;**83**:983–991.
- Azhar S, Luo Y, Medicherla S, Reaven E. Upregulation of selective cholesteryl ester uptake pathway in mice with deletion of low-density lipoprotein receptor function. *J Cell Physiol* 1999;**180**:190–202.
- Calvo D, Vega MA. Identification, primary structure, and distribution of CLA-I, a novel member of the CD36/LIMPII gene family. *J Biol Chem* 1993;**268**:18929–18935.
- Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. *J Lipid Res* 1995;**36**:211–228.
- Jimenez LM, Binelli M, Bertolin K, Pelletier RM, Murphy BD. Scavenger receptor B-I and luteal function in the mouse. *J Lipid Res* 2010;**51**:2362–2371.
- Khorram O, Han G. Influence of progesterone on endometrial nitric oxide synthase expression. *Fertil Steril* 2009;**91**:2157–2162.
- Kolmakova A, Wang J, Brogan R, Chaffin C, Rodriguez A. Deficiency of the scavenger receptor class B type I negatively affects progesterone secretion in human granulosa cells. *Endocrinology* 2010;**151**:5519–5527.
- Landschulz KT, Pathak RK, Rigotti A, Krieger M, Hobbs HH. Regulation of scavenger receptor, class B, type I, a high density lipoprotein receptor, in liver and steroidogenic tissues of the rat. *J Clin Invest* 1996;**98**:984–995.
- Li XA, Titlow WB, Jackson BA, Giltiy N, Nikolova-Karakashian M, Uittenbogaard A, Smart EJ. High density lipoprotein binding to scavenger receptor, Class B, type I activates endothelial nitric-oxide synthase in a ceramide-dependent manner. *J Biol Chem* 2002;**277**:11058–11063.
- McCarthy JJ, Lewitzky S, Reeves C, Permutt A, Glaser B, Groop LC, Lehner T, Meyer JM. Polymorphisms of the HDL receptor gene associated with HDL cholesterol levels in diabetic kindred from three populations. *Hum Hered* 2003a;**55**:163–170.
- McCarthy JJ, Lehner T, Reeves C, Moliterno DJ, Newby LK, Rogers WJ, Topol EJ. Association of genetic variants in the HDL receptor, SR-BI, with abnormal lipids in women with coronary artery disease. *J Med Genet* 2003b;**40**:453–458.
- McCarthy JJ, Somji A, Weiss LA, Steffy B, Vega R, Barrett-Connor E, Talavera G, Glynne R. Polymorphisms of the scavenger receptor class B member I are associated with insulin resistance with evidence of gene by sex interaction. *J Clin Endocrinol Metab* 2009;**94**:1789–1796.
- McConihay JA, Honkomp AM, Granholm NA, Woollett LA. Maternal high density lipoproteins affect fetal mass and extra-embryonic fetal tissue sterol metabolism in the mouse. *J Lipid Res* 2000;**41**:424–432.
- Miettinen HE, Rayburn H, Krieger M. Abnormal lipoprotein metabolism and reversible female infertility in HDL receptor (SR-BI)-deficient mice. *J Clin Invest* 2001;**108**:1717–1722.
- Naj AC, West M, Rich SS, Post W, Kao WH, Wasserman BA, Herrington DM, Rodriguez A. Association of scavenger receptor class B type I polymorphisms with subclinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis. *Circ Cardiovasc Genet* 2010;**3**:47–52.
- Piccinni M-P, Beloni L, Livi C, Maggi E, Scarselli G, Romagnani S. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. *Nat Med* 1998;**4**:1020–1024.
- Plaino L, Stomati M, Casarosa E, Artini PG, Santuz M, D'Ambrogio G, Cobellis L, Luisi M, Genazzani AR, Petraglia F. Ovarian follicular fluid contains immunoreactive estriol: lack of correlation with estradiol concentrations. *Gynecol Endocrinol* 2000;**14**:231–235.
- Ramachandran S, Song M, Murphy AA, Parthasarathy S. Expression of scavenger receptor class B1 in endometrium and endometriosis. *J Clin Endocrinol Metab* 2001;**86**:3924–3928.
- Reaven E, Tsai L, Azhar S. Cholesterol uptake by the 'selective' pathway of ovarian granulosa cells: early intracellular events. *J Lipid Res* 1995;**36**:1602–1617.
- Rigotti A, Trigatti BL, Penman M, Rayburn H, Herz J, Krieger M. A targeted mutation in the murine gene encoding the high density lipoprotein (HDL) receptor scavenger receptor class B type I reveals its key role in HDL metabolism. *Proc Natl Acad Sci USA* 1997;**94**:12610–12615.
- Rigotti A, Miettinen HE, Krieger M. The role of the high-density lipoprotein receptor SR-BI in the lipid metabolism of endocrine and other tissues. *Endocr Rev* 2003;**24**:357–387.
- Roberts CGP, Shen H, Mitchell BD, Damcott CM, Shuldiner AR, Rodriguez A. Variants in scavenger receptor class B type I gene are associated with HDL cholesterol levels in younger women. *Hum Hered* 2007;**64**:107–113.
- Rodriguez-Esparragon F, Rodriguez-Perez JC, Hernandez-Trujillo Y, Macias-Reyes A, Medina A, Caballero A, Ferrario CM. Allelic variants of the human scavenger receptor class B type I and paraoxonase I on coronary heart disease: genotype-phenotype correlations. *Arterioscler Thromb Vasc Biol* 2005;**25**:854–860.
- Seetharam D, Mineo C, Gormley AK, Gibson LL, Vongpatanasin W, Chambliss KL, Hahner LD, Cummings ML, Kitchens RL, Marcel YL et al. High-density lipoprotein promotes endothelial cell migration and reendothelialization via scavenger receptor-B type I. *Circ Res* 2006;**98**:63–72.
- Swarnakar S, Temel RE, Connelly MA, Azhar S, Williams DL. Scavenger receptor class B type I mediates selective uptake of low density lipoprotein cholesteryl ester. *J Biol Chem* 1999;**274**:29733–29739.
- Trigatti B, Rayburn H, Vinals M, Braun A, Miettinen H, Penman M, Hertz M, Schrenzel M, Amigo L, Rigotti A et al. Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. *Proc Natl Acad Sci USA* 1999;**96**:9322–9327.
- Velasco M, Alexander C, King J, Zhao Y, Garcia J, Rodriguez A. Association of lower plasma estradiol levels and low expression of scavenger receptor class B type I in infertile women. *Fert Steril* 2006;**85**:1391–1397.
- Watanabe K, Shin EK, Hashino M, Tachibana M, Watarai M. Toll-like receptor and class B scavenger receptor type I are required for bacterial uptake by trophoblast giant cells. *Mol Immunol* 2010;**47**:1989–1996.
- West M, Greason E, Kolmakova A, Jahangiri A, Asztalos B, Pollin TI, Rodriguez A. Scavenger receptor class B type I protein as an independent predictor of HDL cholesterol levels in subjects with hyperalphalipoproteinemia. *J Clin Endo Metab* 2009;**94**:1451–1457.
- Woollett LA. Where does fetal and embryonic cholesterol originate and what does it do? *Annu Rev Nutr* 2008;**28**:97–114.
- Yesilaltay A, Morales MG, Amigo L, Zanlungo S, Rigotti A, Karackattu SL, Donahee MH, Kozarsky KF, Krieger M. Effects of hepatic expression of the high-density lipoprotein receptor SR-BI on lipoprotein metabolism and female fertility. *Endocrinology* 2006;**147**:1577–1588.