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

Melissa Yates1, Antonina Kolmakova2, Yulian Zhao1, and Annabelle Rodriguez2,*

1Department of Gynecology and Obstetrics, Division of Reproductive Endocrinology, Johns Hopkins University School of Medicine, Baltimore, MD, USA 2Department of Medicine, Division of Endocrinology, Johns Hopkins University School of Medicine, Baltimore, MD, USA

*Correspondence address. Johns Hopkins Bayview Medical Center, Division of Endocrinology and Metabolism, Mason F. Lord Building, Center Tower, Room 639, 5200 Eastern Avenue, Baltimore, MD 21224, USA. Tel: +1-410-550-8487; Fax: +1-410-550-3219; E-mail: arodrig5@jhmi.edu

Submitted on November 18, 2010; resubmitted on March 9, 2011; accepted on March 23, 2011

**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 ± 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 3β-hydroxysteroid dehydrogenase (3β-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 retrieval. Overall, our results showed that the non-synonymous SNP, rs4238001, was significantly associated with poor fetal outcome in the entire group, rs4238001 was significantly associated with lower follicular progesterone levels, while we previously showed that this SNP was associated with higher follicular progesterone levels. 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. L.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 μl 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 N2. 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.

## 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 stimulation 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 QIAamp DNA 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.

## 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 χ² 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
The study population consisted of 274 adult women with a mean age of 36.4 ± 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), 3% Hispanic, (n = 14) and 2% other (n = 6). Other general characteristics of the entire group included a mean BMI of 26.8 ± 7.1 (range 16.8–53.3), and previous IVF attempts of 1.8 ± 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 higher follicular progesterone levels compared with subjects homozygous for the major C allele (homozygous major CC: 5061 ± 285 nmol/l; heterozygous CT: 5367 ± 260; homozygous minor TT: 6498 ± 462) (P = 0.03) (Fig. 1B). In the Caucasian group, carriers of the minor allele for rs4238001 had lower follicular progesterone levels (2528 ± 1517) when compared with homozygous carriers of the major allele (5629 ± 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 ± 450 nmol/l; heterozygous CT: 5346 ± 359; homozygous minor TT: 7041 ± 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.

**Results**

The study population consisted of 274 adult women with a mean age of 36.4 ± 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), 3% Hispanic, (n = 14) and 2% other (n = 6). Other general characteristics of the entire group included a mean BMI of 26.8 ± 7.1 (range 16.8–53.3), and previous IVF attempts of 1.8 ± 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 ± 285 nmol/l; heterozygous CT: 5367 ± 260; homozygous minor TT: 6498 ± 462) (P = 0.03) (Fig. 1B). In the Caucasian group, carriers of the minor allele for rs4238001 had lower follicular progesterone levels (2528 ± 1517) when compared with homozygous carriers of the major allele (5629 ± 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 ± 450 nmol/l; heterozygous CT: 5346 ± 359; homozygous minor TT: 7041 ± 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.
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 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.,...
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 steriodogenic enzymes such as P450sc and 3β-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 → 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), 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 P450sc and 3β-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, P450sc and 3β-HSD (Kolmakova et al., 2010). Moreover, Piccinni et al. (1998) have shown the importance of progesterone in acting as an immuno-suppressant by activation of TH2 helper cells. Thus while normal human endometrium has been shown to have low RNA expression levels of StAR, SCC and 3β-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

**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, $P = 0.03$.
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, protocol (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.

Acknowledgements
We thank the staff at the Johns Hopkins Fertility Center for help with the study logistics.

Funding
The study was supported by a Burroughs Wellcome Clinical Scientist Award in Translational Research to Dr Annabelle Rodriguez.

References


