BIOLOGY OF REPRODUCTION (2014) **91**(5):117, 1–10
Published online before print 13 August 2014.
DOI 10.1095/biolreprod.114.119883

Minireview

Genetic Alterations Affecting Cholesterol Metabolism and Human Fertility[1]

Anthony M. DeAngelis,[3] Meaghan Roy-O'Reilly,[4] and Annabelle Rodriguez[2,5]

[3]*University of Connecticut Health Center, Farmington, Connecticut*
[4]*University of Connecticut School of Medicine, Farmington, Connecticut*
[5]*Center for Vascular Biology, University of Connecticut Health Center, Farmington, Connecticut*

ABSTRACT

Single nucleotide polymorphisms (SNPs) represent genetic variations among individuals in a population. In medicine, these small variations in the DNA sequence may significantly impact an individual's response to certain drugs or influence the risk of developing certain diseases. In the field of reproductive medicine, a significant amount of research has been devoted to identifying polymorphisms which may impact steroidogenesis and fertility. This review discusses current understanding of the effects of genetic variations in cholesterol metabolic pathways on human fertility that bridge novel linkages between cholesterol metabolism and reproductive health. For example, the role of the low-density lipoprotein receptor (LDLR) in cellular metabolism and human reproduction has been well studied, whereas there is now an emerging body of research on the role of the high-density lipoprotein (HDL) receptor scavenger receptor class B type I (SR-BI) in human lipid metabolism and female reproduction. Identifying and understanding how polymorphisms in the *SCARB1* gene or other genes related to lipid metabolism impact human physiology is essential and will play a major role in the development of personalized medicine for improved diagnosis and treatment of infertility.

cholesterol metabolism, genetics, infertility, lipoproteins, mutation, polymorphism, reproduction

INTRODUCTION

Infertility represents a serious health problem affecting approximately 15% of reproduction-aged couples in the United States and is defined as the failure to achieve a successful pregnancy after 12 or more months of appropriate, timed, unprotected intercourse or therapeutic donor insemination [1–4]. According to the American Society for Reproductive Medicine, approximately 30% of infertility cases can be attributed to female causes and 30% to male factors, and the remaining cases involve a combination of male and female factors in addition to unexplained causes [5]. Understanding the various pathologic mechanisms underlying infertility represents an active area of research and serves as an important first step in our ability to treat this health problem.

Genetic mutations leading to functional alterations in proteins involved in the steroidogenic pathway have been shown to adversely impact human fertility. Moreover, research of the molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders as they relate to fertility have been well characterized and thoroughly reviewed [6–11].

An often overlooked, but important, area of research relates to genetic mutations affecting cholesterol metabolism and their impact on human fertility. Cholesterol serves as an essential substrate for steroid hormone production and membrane synthesis. Genetic mutations altering the function of proteins involved in cholesterol uptake, mobilization from stored intracellular pools, and synthesis significantly alter normal cellular function and, in turn, impact fertility. This review summarizes some of these known genetic mutations. In particular, we highlight work focused on the functional impact of genetic mutations involved in cholesterol uptake, mobilization, and de novo cholesterol synthesis and review how these alterations can affect cholesterol metabolism and human fertility.

CHOLESTEROL METABOLISM

Cholesterol is essential for plasma membrane synthesis and fluidity. In steroidogenic cells of the gonads, adrenal glands, and placenta, cholesterol also acts as a necessary precursor for the biosynthesis of steroid hormones, which in turn, regulate vitally important physiologic functions including but not limited to the control of reproductive pathways, development of secondary sexual characteristics, and salt balance [12, 13]. Activated steroidogenic cells demand high levels of cholesterol substrate. Several mechanisms within the cell work in a coordinated manner to ensure adequate replenishment and availability of the cholesterol pool. These major mechanisms include receptor-mediated uptake of lipoprotein-derived cholesterol (i.e., low-density lipoprotein receptor [LDLR] and/or scavenger receptor class B type I [SR-BI]), hydrolysis of stored intracellular cholesteryl esters by hydrolases such as hormone-sensitive lipase, and de novo cholesterol synthesis by a multistep process using acetate as the starting substrate (Fig. 1).

Most species, including humans, depend mostly on cellular uptake of lipoprotein cholesterol as the major precursor for steroidogenesis [14, 15]. There are various mechanisms that

[1]Supported by a Burroughs Wellcome Clinical Translational Award and National Institutes of Health grant HL075646 to A.R. Inventorship rights to a molecular diagnostic test relevant to SCARB1, rs4238001 (REFSEQ accession number: NP_001076428.1), belong to A.R.
[2]Correspondence: Annabelle Rodriguez, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030. E-mail: rodriguezoquendo@uchc.edu

Received: 1 April 2014.
First decision: 27 April 2014.
Accepted: 7 August 2014.

eISSN: 1529-7268 http://www.biolreprod.org
ISSN: 0006-3363

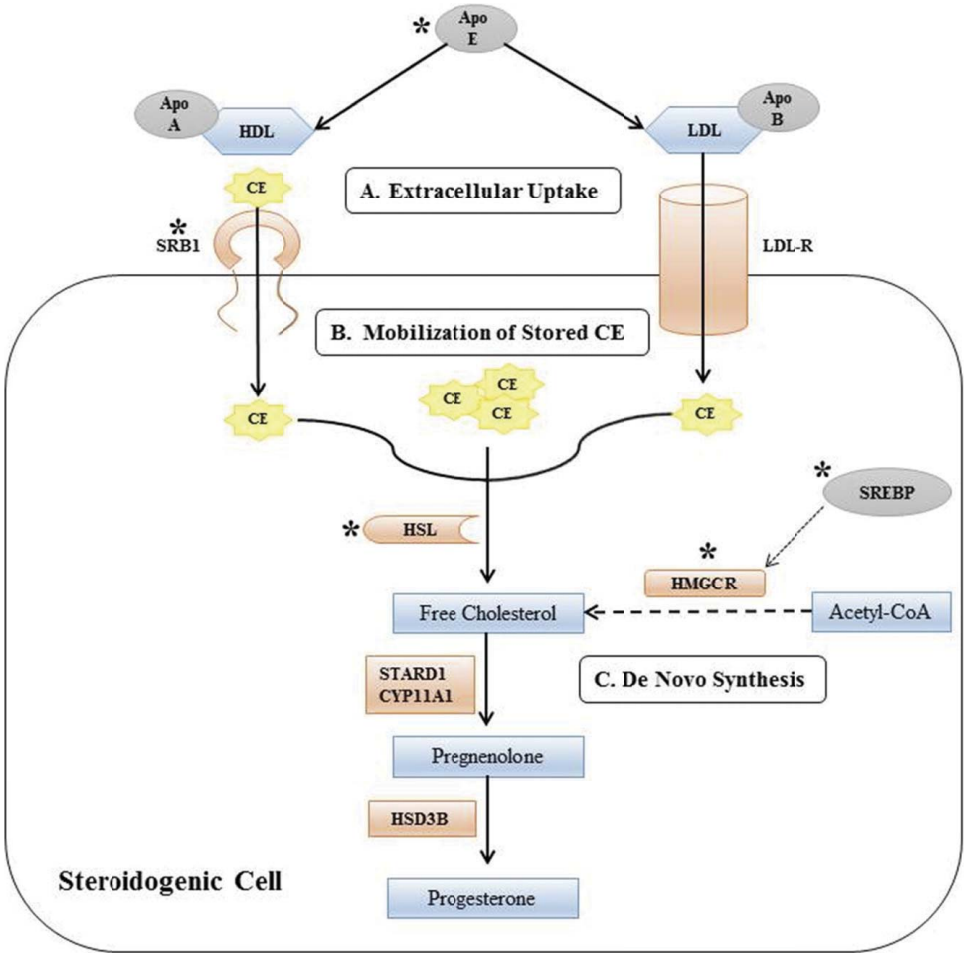

FIG. 1. Sources of cellular cholesterol. The three pathways by which cells maintain an adequate intracellular cholesterol pool are outlined here, including uptake (**A**), mobilization (**B**), and de novo synthesis (**C**). Asterisks denote the location of SNPs discussed in this review. ApoA = apolipoprotein A; CYP11A1 = cytochrome P450, family 11, subfamily A, polypeptide 1; HSD3B = 3 beta-hydroxysteroid dehydrogenase; STARD1 = steroidogenic acute regulatory protein D1.

facilitate cholesterol uptake into a cell. The two dominant mechanisms include uptake via the LDLR [15–17] and selective cholesteryl ester uptake mediated by SR-BI [18]. LDLR-mediated uptake involves the binding of apolipoprotein B (ApoB)- and ApoE-enriched cholesterol particles with subsequent endocytosis of the LDL receptor and uptake of the cholesterol-containing lipoprotein particles into the cell. In contrast, SR-BI mediates high-capacity selective uptake of cholesteryl esters from the core of high-density lipoprotein (HDL), LDL, and very-low-density lipoprotein (VLDL) lipoproteins [19]. Selective uptake refers to the process whereby lipoproteins dock on the extracellular loop of SR-BI (Fig. 2), and the cholesteryl esters within the core of the lipoprotein are transferred into the interior of the cell via a channel formed by dimerization of SR-BI receptors. The lipoprotein, now depleted of cholesteryl esters, dissociates from the receptor and is not internalized. The internalized cholesteryl ester is then hydrolyzed by hydrolases such as hormone-sensitive lipase to generate free or unesterified cholesterol (UC), which is then used for cellular processes such as steroidogenesis or membrane integrity [20].

Following stimulation by tropic hormones, cholesterol may be recruited from the hydrolysis of stored cytoplasmic cholesteryl esters. This process involves the participation of hormone-sensitive lipase (HSL), an enzyme expressed in steroidogenic tissues and which functions to convert stored cholesteryl esters to UC [21]. This mechanism generates UC for the immediate synthesis of steroid hormones following cellular activation [22].

Intracellular cholesterol may also be derived via de novo synthesis in the endoplasmic reticulum (ER) from acetyl-coenzyme A (CoA), a process regulated by the enzyme 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) [23–25]. The contribution of this pathway in providing cholesterol substrate varies among species, is heavily influenced by the amount of intracellular cholesterol, and is regulated by the activation of the sterol regulatory element-binding proteins (SREBPs). When cells sense abundant intracellular cholesterol stores, the de novo cholesterol synthetic pathway remains inactive or quiescent. During this time, immature or large SREBPs remains complexed with two other proteins, SREBP cleavage-activating protein (SCAP) and INSIG1, which prevent its translocation to the nucleus. Following a decrease in the intracellular cholesterol pool, as seen in stimulated steroidogenic cells, which in turn would signal a demand for more intracellular cholesterol, there is dissociation of INSIG1 from the complex and subsequent mobilization, cleavage, and translocation of SREBP to the nucleus. In the nucleus, SREBP functions as a transcription factor, resulting in upregulation of the LDL receptor and HMG-CoA reductase, leading to both increased plasma membrane

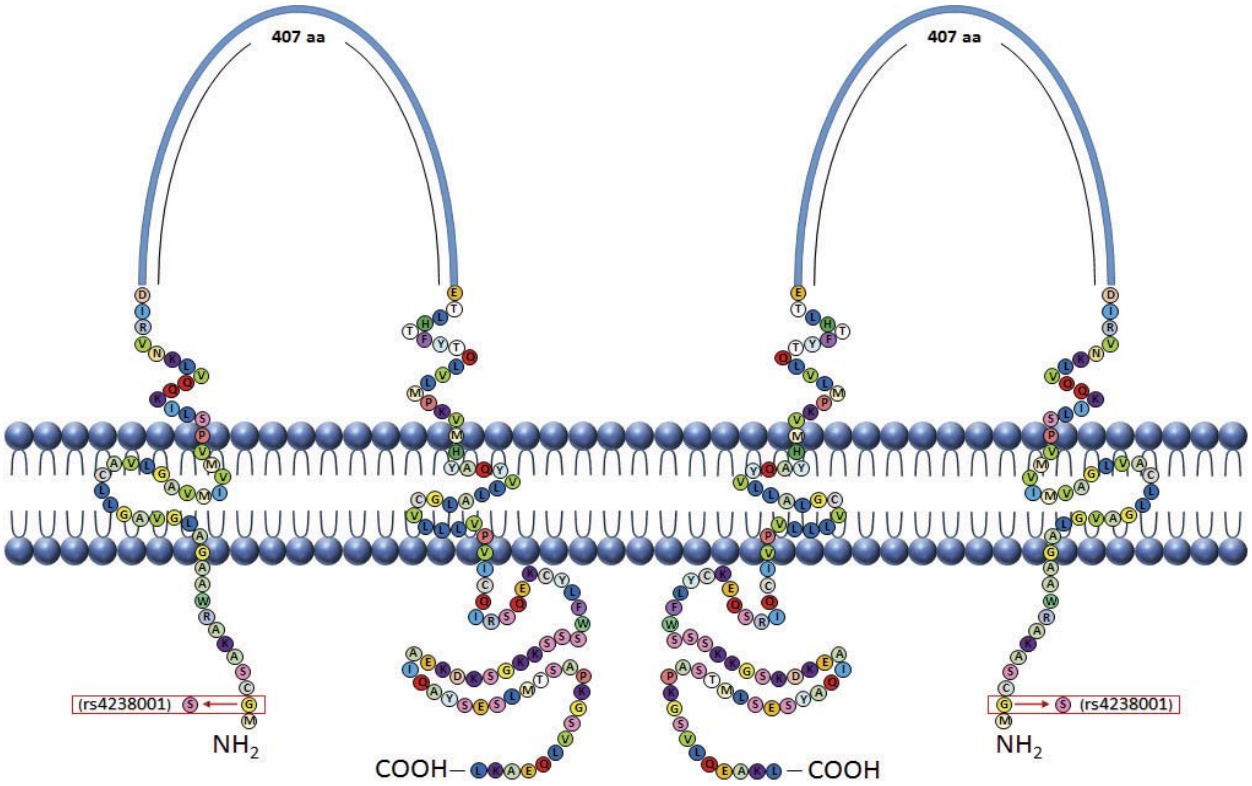

FIG. 2. Structure of the scavenger receptor class B type 1 (SR-BI). SR-BI is a receptor that mediates high-capacity selective uptake of cholesteryl esters from the core of HDL, LDL and VLDL via lipoprotein docking on the extracellular loop. A deficiency in SR-BI is associated with infertility, and the location of the rs4238001 SNP discussed in this review is highlighted here.

receptor-derived cholesterol uptake and de novo cholesterol production [26, 27].

GENETIC ALTERATIONS AFFECTING CHOLESTEROL UPTAKE AND THEIR INFLUENCE ON HUMAN FERTILITY

Lipoprotein particles serve as vehicles for cholesterol transport throughout the body and function to regulate intracellular cholesterol levels within a tight physiologic range, as an excess or insufficiency is typically deleterious to normal cellular function [28, 29].

Lipoprotein-mediated cholesterol uptake is an important mechanism by which cells obtain cholesterol for steroidogenesis. In fact, extracellular uptake has been shown to provide approximately 80% or more of cholesterol substrate for steroidogenic cells of the adrenal glands, corpus luteum, and placenta, with the other 20% supplied by de novo cholesterol synthesis from acetate and other precursors and cholesteryl ester hydrolysis [30–32]. In humans, LDLs, which are composed of the major apoproteins ApoB100 and ApoE as ligands to the LDLR, bind to the LDLR, and this is followed by endocytosis, which leads to internalization of the lipoprotein and the LDLR. In contrast, cholesterol-mediated uptake by SR-BI occurs by selective cholesteryl ester uptake and not by endocytosis. Several known polymorphisms in *APOE* have been discovered and found to significantly impact cholesterol metabolism and human fertility.

Apolipoprotein E

ApoE is a constituent apoprotein of VLDL, intermediate density lipoproteins (IDL), HDL, and chylomicron particles. It is produced in the liver and in macrophages and serves an important role in lipid transport by mediating the binding of lipoprotein particles to their receptors [33]. ApoE-mediated delivery of cholesterol represents an important mechanism by which steroidogenic cells obtain cholesterol for steroid hormone production.

In rodents, deletion of the *Apoe* gene results in the development of severe hypercholesterolemia and decreased expression of the steroidogenic enzymes cytochrome P450 aromatase (Cyp19a1) and 3β-hydroxysteroid dehydrogenase (3βHSD), essential enzymes involved in the biosynthetic pathway of progesterone and estrogen, leading to a decrease in the level of both hormones [34]. In that study, Zhang et al. [34] showed significant alterations in the ER ultrastructure of *Apoe* null mice, which are described as swollen and tubby, and we speculate that these ER changes contributed to the reduced expression and function of Cyp19a1 and 3βHSD. Interestingly, no differences between the fertility rates of *Apoe* null and those of wild-type control mice were observed. However, *Apoe* null mice did possess greater numbers of ovarian follicles as well as an increased ratio of ovarian follicle atresia and apoptosis than their wild-type counterparts [34].

Three important polymorphisms have been discovered in the human *APOE* gene [35, 36]. These codominant alleles, designated ε2, ε3, and ε4, give rise to 3 functionally distinct proteins: ApoE2, ApoE3, and ApoE4 [33], which vary in amino acid residues at positions 112 and 158. ApoE2 is characterized by 2 cysteine residues, ApoE3 by a cysteine and arginine residue, and ApoE4 by 2 arginine residues at these sites [37]. Although these genetic polymorphisms represent small changes in the DNA sequence, studies have found that they exert a significant impact on the functional properties of their corresponding proteins and result in the alteration of

plasma cholesterol and apolipoprotein levels [33] and marked differences in reproductive efficiency [38–41].

In one study, Corbo et al. [40] screened 160 European women to investigate the possible impact of *APOE* polymorphisms on reproductive efficiency. Investigators discovered that women harboring the ε3/ε3 genotype exhibited higher reproductive efficiency, whereas carriers of the ε2 allele had lower numbers of children and pregnancies [40]. *APOE* polymorphisms have also been found to impact male fertility. Setarehbadi et al. [42] found significant differences between the distribution of *APOE* allelic combinations in fertile men and that in infertile men, with a higher percentage of fertile males possessing the ε3/ε3 genotype. This study mirrors the results of Gerdes et al. [41], who reported higher birth rates for children from fathers possessing the ε3/ε3 genotype than from fathers possessing other allelic variations.

Overall, these studies suggested that lower reproductive efficiency is associated with the ε2 allele and higher reproductive efficiency with the ε3 allele.

LDL Receptor

The LDL receptor is an important protein that functions to mediate the uptake of LDL cholesterol, the preferred substrate for steroid hormone production [43–47]. Given the important role the LDL receptor plays in providing adequate substrate for steroidogenesis, one might assume that alterations in its function would lead to significant impairment of fertility. However, LDL receptor-deficient mice are fertile and produce normal sized litters despite exhibiting an altered lipid profile [48]. In contrast, a deficiency of SR-BI is associated with infertility [49, 50]. The ability of SR-BI to bind LDL cholesterol may partially explain the normal fertility exhibited by LDL receptor null mice. To date, no significant polymorphisms have been discovered in the LDL receptor which adversely impact human fertility. A recent publication by Rinninger et al. [51] showed that levels of hepatic and adrenal SR-BI protein expression were not different between wild-type and LDLR knockout mice.

Scavenger Receptor Class B Type I

SR-BI has been identified as the primary lipoprotein receptor for HDL, but it also has the capability to bind LDL, VLDL, and other ligands with high affinity [52–56]. SR-BI is highly expressed in liver, adrenal glands, and ovarian tissues [50], where it plays an important role in mediating selective cholesteryl ester (CE) uptake [20]. This process serves as another lipoprotein receptor-mediated mechanism for providing cholesterol substrate to steroidogenic cells [12, 57–63]. In addition, SR-BI has been shown to mediate the bidirectional movement of UC down a concentration gradient between lipoproteins and cells [64–66].

Much of our knowledge of SR-BI function comes from animal studies. In rat ovaries, SR-BI mRNA is present in interstitial cells, thecal cells, corpora lutea, and luteinized granulosa cells [67]. SR-BI protein expression is upregulated after stimulation with tropic hormones [68, 69] and is associated with an increase in CE uptake [70]. SR-BI expression in ovarian tissue plays a particularly important role in female fertility, as substantial amounts of HDL, but not LDL, are present in follicular fluid surrounding oocytes in ovarian follicles [46, 71–75]. LDL is absent from the follicular fluid of pre-ovulatory follicles because particles range in size from 18 to 23 nm, with a molecular mass of 2.3 million daltons (Da), precluding movement across the basement membrane (size limit, approximately 400 000 Da). [74–77]. In contrast, HDL particles range in size from 8.2 to 13 nm with molecular mass of 200 000 to 400 000 [76, 77]. Due to the absence of other lipoprotein particles in the follicular fluid, SR-BI delivery of HDL may serve as an important mechanism by which cholesterol is delivered to the oocyte and its surrounding steroidogenic support cells. It is not surprising then that genetic alterations in SR-BI have been shown to adversely impact cholesterol metabolism, including that of the ovaries, resulting in alterations in fertility.

Genetically engineered mice lacking SR-BI demonstrate the importance of this protein in reproduction. Female homozygous SR-BI knockout mice are infertile [18, 49, 50], most likely secondary to impaired embryonic development of the offspring, as embryos from SR-BI null females have been shown to develop an abnormal morphology and to arrest in culture. Similar abnormalities were found in unfertilized oocytes, but estrus cycles, progesterone levels, and ovulatory patterns in SR-BI null mice were found to be normal [50]. However, a more recent study by Jimenez et al. [78] found that SR-BI null mice have 50% lower plasma progesterone levels than wild-type controls, as well as ovaries with small corpora lutea, large follicles with hypertrophied theca cells, and follicular cysts with blood-filled cavities. These later data are more consistent with the phenotypic data observed in humans carrying a single nucleotide polymorphism (SNP) in the *SCARB1* gene, which encodes the SR-BI protein. As a result of altered SR-BI function, null mice have elevated and abnormally large ApoE-enriched circulating HDL particles as well as an elevated UC:total cholesterol ratio (known as the UC:TC ratio), a phenotype which is most likely secondary to defective removal of the core CE from these lipoprotein particles [79].

Interestingly, fertility in SR-BI null mice can be rescued by methods that act to restore normal cholesterol metabolism. Miettinen et al. [49] treated SR-BI null mice with the HDL cholesterol-lowering drug probucol [80, 81]. They found that this treatment reduced and remodeled the abnormally large HDL particles and restored fertility. In addition, these investigators also performed bilateral ovarian transplantation in which ovaries were removed from SR-BI null mice and subsequently transplanted into ovariectomized SR-BI-positive hosts. The transplanted SR-BI-negative ovaries began functioning normally in the SR-BI-positive environment and developed the capacity to produce functional oocytes, resulting in restoration of fertility. In support of these findings, Yesilaltay et al. [79] confirmed the presence of abnormally large HDL particles and an elevated UC:TC ratio in SR-BI null mice. Investigators demonstrated that hepatic restoration of SR-BI expression alone via adenoviral transduction or stable transgenesis in SR-BI null mice substantially reduced levels of abnormally large HDL, normalized the UC:TC ratio, and restored fertility [79]. Taken together, these data led the investigators to hypothesize that infertility in SR-BI null mice is not due to irreversible defects in oocyte or embryonic development but rather secondary to abnormal lipoprotein metabolism and/or the abnormal transfer of cholesterol to or from the oocyte or supporting cells.

As in rodents, alterations in human SR-BI function have been shown to adversely impact cholesterol metabolism and fertility. In vitro experiments in the HGL-5 immortalized human granulosa cell line, in which SR-BI had been knocked down using interfering RNA, resulted in significantly reduced progesterone secretion after stimulation with forskolin (an activator of cyclic AMP) compared to that in treated controls [82]. This is in line with recent rodent data by Jimenez et al.

[78], which reported a 50% reduction in circulating plasma levels of progesterone in SR-BI null mice.

Several SNPs in the *SCARB1* gene have been identified and found to impact cholesterol metabolism and human fertility [83] (Fig. 1). In a prospective study of adults with hyperalphalipoproteinemia (defined as subjects with fasting HDL-C levels of >60 mg/dl), West et al. [84] found that certain polymorphisms in *SCARB1* were associated with increased SR-BI protein degradation that resulted in lower receptor protein expression. In addition, these expression levels were inversely proportional to HDL lipoprotein size and positively associated with CE uptake from HDL [84]. In another study, Yates et al. [85] evaluated the association of *SCARB1* polymorphisms and fertility outcomes in women undergoing in vitro fertilization. In that study, investigators found the missense rs4238001 (REFSEQ accession number: NP_001076428.1) SNP (encoding an amino acid change from glycine to serine at amino acid 2) was significantly associated with lower follicular fluid progesterone levels and poor fetal viability [85]. Finally, a study by Velasco et al. [86] found that a cohort of women undergoing in vitro fertilization treatment exhibited low SR-BI-RNA expression, a finding which was associated with lower baseline and peak estradiol levels and lower number of retrieved and fertilized oocytes than in women expressing higher levels of SR-BI [86]. Taken together, research in both animal models and human studies has demonstrated the importance of SR-BI and its role in cholesterol metabolism and fertility.

GENETIC ALTERATIONS AFFECTING CHOLESTEROL MOBILIZATION AND THEIR INFLUENCE ON HUMAN FERTILITY

The ability of the cell to store and mobilize intracellular cholesterol is the result of intricate mechanisms which work to regulate the size and availability of the cholesterol pool. Following uptake, UC can be esterified by acyl CoA:cholesterol acyl transferase (ACAT) and stored as CE in intracellular lipid droplets for later use [87]. Following stimulation with tropic hormones, stored CE can be hydrolyzed by HSL, mobilized to the mitochondria, and subsequently used as a substrate for immediate synthesis of steroid hormones [22, 88]. SNPs in HSL have been discovered and found to impact cholesterol metabolism and fertility.

Hormone-Sensitive Lipase

HSL is an important protein with multiple physiological functions. It plays an essential role in lipid metabolism and is responsible for mediating hydrolysis of diacylglycerol and triacylglycerol [21]. In addition, it mediates the hydrolysis of cholesteryl esters in steroidogenic tissues of the adrenal glands, ovaries, and testis, providing substrate for the immediate synthesis of steroid hormones [89, 90].

Disruption of HSL in mice results in an altered metabolic phenotype. HSL null mice exhibit increased hepatic insulin sensitivity and are resistant to diet-induced obesity [91, 92]. With regard to cholesterol metabolism and fertility, the testes of HSL null mice were found to completely lack neutral cholesteryl ester hydrolase activity, resulting in increased accumulation of intracellular CE. This finding was associated with oligospermia with reduced motility and a consequential reduction in fertility [93, 94]. Vallet-Erdtmann et al. [95] found that reintroduction of HSL into the testis of HSL null mice reversed CE accumulation and fully restored fertility.

In humans, several polymorphisms in the *HSL* gene have been identified [96–100]. The C-60 G polymorphism is an *HSL* promoter variant that is associated with a 40% reduction in promoter activity in vitro [99]. A study by Vatannejad et al. [101] observed significant differences between the *HSL* genotype distribution in fertile male mice and that in infertile males ($n = 164$, $n = 169$, respectively). Interestingly, the CC genotype conferred a 2.4-fold greater risk for male infertility than for male carriers of the GC or GG genotype [101].

Taken together, these data suggest that HSL mobilization of CE plays an essential role in the process of spermatogenesis and that disruption of HSL function adversely impacts fertility.

GENETIC ALTERATIONS AFFECTING DE NOVO CHOLESTEROL SYNTHESIS AND THEIR INFLUENCE ON HUMAN FERTILITY AND EMBRYONIC VIABILITY

De novo synthesis represents another major source of cellular cholesterol, which is required for structural integrity, steroidogenesis, and oocyte maturation. We refer readers to a recent comprehensive review of malformations caused by enzymatic defects in cholesterol synthesis [102], and we would like to highlight some of these major enzymatic defects that result in embryonic lethality and/or developmental abnormalities.

As shown in Figure 3, there are many enzymatic steps in the conversion of acetate to cholesterol, and multiple enzymatic defects have been reported, particularly involving steps downstream of squalene production [102].

HMGCoA Reductase

3-Hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is the rate-limiting enzyme in the early steps of cholesterol synthesis and acts to catalyze the formation of mevalonate from 3-hydroxy-3-methyglutaryl coenzyme A. In 2003, Ohashi et al. [103] showed that mouse embryos genetically engineered to have complete deficiency of *Hmgcr* did not develop past the blastocyst stage; thus, no viable *Hmgcr* null mice were produced from heterozygotic crossings. Investigators determined that pre-implantation embryos (embryonic day 3.5 [E3.5]) appeared to be normal and that the defect in viability likely occurred at the level of implantation. An attempt to infuse replacement mevalonate via a subcutaneous osmotic pump did not overcome the defect in *Hmgcr* null embryos, as the embryos were able to implant but did not progress to viable offspring. Mice heterozygous for this mutation appeared to be normal in development, gross anatomy, fertility, and capacity for cholesterol synthesis despite decreased HMGCR activity. Overall cholesterol biosynthesis remained unchanged in the liver of these mice, and lipoprotein profiles were similar to those of wild-type mice profiles, suggesting that one copy of the *Hmgcr* gene produces sufficient HMGCR for hepatic cholesterol synthesis.

HMGCR is regulated both transcriptionally and post-transcriptionally. It is well established that when cells sense a reduction in intracellular cholesterol mass, activation of the ER-residing SREBPs (1a, 1c, and 2) occurs, which leads to its transformation from a larger ER protein to a smaller nuclear transcription factor [104]. Cholesterol genes known to be activated by SREBP2 include *HMGCR*, *LDLR*, and *INSIG*, among others [105]. Complete knockout of SREBP2 in mice is 100% embryonically lethal [104].

Surprisingly, little is known regarding the functional effect of variants in the SREBP2 gene in relation to reproduction or other medical diseases. Liu et al. [106] reported a significant

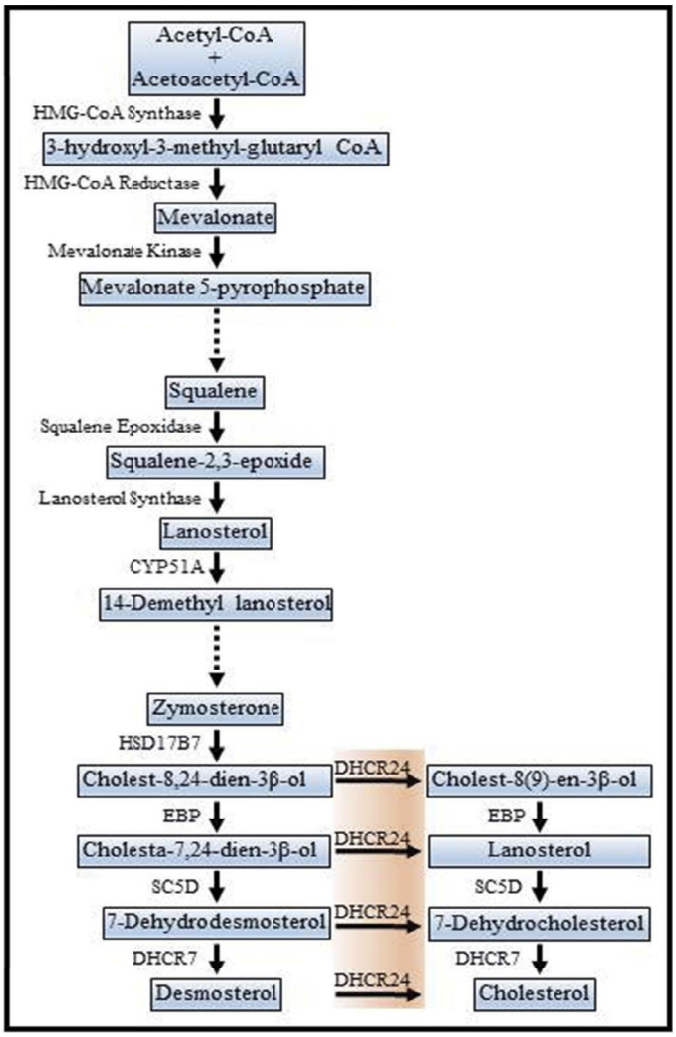

FIG. 3. De Novo cholesterol synthesis. The enzymatic steps in the conversion of acetate to cholesterol are outlined here. Mutations in these enzymes have been shown to result in impaired embryonic viability.

increase in LDL-cholesterol levels in Chinese Han female carriers of the CC genotype for the rs4822063 SREBP2 variant. Incrementally more is known regarding the functional effects of variations in the *HMGCR* gene in human reproduction. In 2007, Steffen et al. [107] examined genetic causes in the mother and infant associated with preterm delivery (PTD). These investigators were particularly interested in examining the effects of genetic variations in cholesterol genes in the mother and infant on the risk of PTD. Cholesterol genes were genotyped in infants born before 37 wk of gestation ($n = 414$) and in at least 1 parent (253 mothers, 351 fathers). Selection of candidate genes was based on a previous publication from Knoblauch et al. [108] which identified haplotypes responsible for most of the genetic variance in LDL, HDL, and LDL/HDL in a German population. Results in the infant dataset showed a significant association of variations in the *APOAI* gene (ApoA-I is the major apolipoprotein in HDL fractions) and *HMGCR* (rs2303152). In the maternal dataset, the *HMGCR* variant rs2303152 was not analyzed due to low allele frequency; however, the results showed a significant association of PTD with A*BCA1*, *LCAT*, *LIPC*, and *APOE*, all genes associated with HDL metabolism. The authors concluded that there might be a benefit in assessing the effect of nutritional risk on PTD by genetic testing for common variations in cholesterol genes.

Lanosterol

Lanosterol represents the first step in sterol formation and can be converted by lanosterol 14 α-demethylase (CYP51, a member of the cytochrome P450 family), to follicular fluid meiosis-activating factor (FF-MAF), a sterol intermediate that has been studied extensively and was shown to activate meiosis in gametes [109]. Recently, Lewinska et al. [110] examined polymorphisms within the *CYP51A1* gene and found significant associations with birth weight and maternal lipid levels. Genotyping for CYP51A1 variants was performed in samples isolated from four different populations of mothers experiencing preterm birth or from neonates affected by growth retardation or preterm birth. In a cohort of women and neonates from Western European descent, the investigators identified 22 sequence variants and reported 10 as novel and rare. Of the polymorphisms identified, the minor allele frequencies (MAF) for the common variants rs6465348 and rs12673910 were significantly higher in neonates and mothers affected by preterm birth than in the general population. Based on its high MAF and its location in the CYP51A1 3′ untranslated region (UTR), the investigators chose rs6465348 for further study and found a significant association between low body weight for gestational age and lower LDL cholesterol and total cholesterol in women. These genomic approaches underscore the effort to identify genetic causes for infertility problems related to alterations in lipid metabolism.

In addition to lanosterol's well-known role in generating meiosis-activating factor, other studies have suggested an intriguing role for lanosterol and dihydrolanosterol (a reduction product of lanosterol) in the post-translational regulation of HMGCoA reductase [105, 111]. As mentioned previously, Insig is an ER-resident protein that forms a complex with SREBP. Song et al. [105] showed that lanosterol enhanced Insig-mediated ubiquitination and degradation of HMGCoA reductase. Lange et al. [111] subsequently showed that 24,25dihydrolanosterol could potently inhibit HMGCoA reductase activity in fibroblasts. Together, these studies strongly suggest that lanosterol exerts a negative feedback inhibition on the rate-limiting enzyme of cholesterol synthesis.

Desmosterol

In the Bloch pathway, the enzyme 3β-hydroxysteroid-Δ24 reductase (DHCR24) catalyzes the conversion of desmosterol in the final steps of cholesterol formation [112]. A recent review by Zerenturk et al. [112] highlights the renewed interest in studying the biological effects of desmosterol. DHCR24 was first identified due to high homology with the *DIMINUTO*/*DWARF1* gene in *Arabidopsis thaliana*; it was also separately identified later as selective Alzheimer's disease indicator 1 (Seladin-1) due to its association with Alzheimer's disease. The *DCHR24* gene is highly expressed in brain, liver, and steroidogenic tissues; missense mutations in this gene lead to desmosterolosis, a rare autosomal recessive disorder characterized by congenital anomalies and elevated desmosterol levels [113].

The first report of a child with congenital malformations and confirmed elevated levels of desmosterol were reported in 1998 by FitzPatrick et al. [113]. The child was born at 34 weeks gestation with extensive developmental abnormalities (including macrocephaly, cleft palate, and short limbs) and died within 1 hour of birth due to respiratory distress. Desmosterol levels were measured using gas chromatography-mass spectrometry (GC-MS) from extracts isolated from liver, kidney, and brain tissues. The highest concentrations of desmosterol were found in the brain tissue of the deceased child, whereas plasma levels

were considered normal in the mother but markedly elevated in the father. The authors also noted that exposure to triparanol, a highly teratogenic inhibitor of DHCR24, results in similar malformations, underlining the importance of this enzyme in human development [113].

More recently, Schaaf et al. [114] reported a third case of desmosterolosis and also examined the functional effects of polymorphisms in the *DCHR24* gene in this case report. These investigators determined that the affected infant was a compound heterozygote for 2 novel missense mutations, c.281G > A (p.R94H, which was inherited from the mother) and c.1438G > A (p.E480K, which was inherited from the father). In yeast expression assays, the two mutations significantly lowered DHCR24 enzymatic activity, as measured by the conversion of desmosterol to cholesterol, compared to that in wild-type DHCR24. Mutations in this infant were not fatal, and a trial of therapy with riboflavin, nicotinamide, and thiamine was initiated, but the investigators reported concerns regarding noncompliance with use of the prescribed treatment. A subsequent analysis of sterol levels at 14 months showed no significant changes in desmosterol or cholesterol levels [109].

Admittedly, the clinical condition of desmosterolosis is rare; however, there have been a number of in vitro studies examining other aspects of desmosterol effects on inflammation and cell proliferation. For instance, McGrath et al. [115] reported an interesting relationship between SR-BI and DHCR24. Using human coronary artery endothelial cells (HCAEC), these investigators incubated the cells with recombinant or native HDLs and found that preincubation of the cells with the HDL particles suppressed NF-κB-mediated DNA transcription. One of the genes transcriptionally regulated by preincubation with HDL was *DHCR24*, which had been shown previously to be a hydrogen peroxide scavenger [116] and have antioxidant properties [117]. Furthermore, after SR-BI expression was silenced in the HCAEC, basal and stimulated mRNA levels of DHCR24 were significantly reduced. These results showed that the effect of DHCR24 in modulating inflammatory responses in endothelial cells was dependent on SR-BI. It is unknown if there is a direct interaction between SR-BI and DHCR24, and this should be explored further.

7-Dehydrocholesterol

Of the enzymatic defects identified in the de novo cholesterol synthesis pathway, the mutation resulting in Smith-Lemli-Opitz syndrome (SLOS) is the best characterized. SLOS is an autosomal recessive disease caused by a defect in the 3β-hydroxysterol Δ7 cholesterol reductase (*DHCR7*) gene leading to reduced cholesterol end-product and accumulation of 7-dehydrocholesterol and 27-hydroxy-7-dehydrocholesterol [118]. This deficiency results in morphological abnormalities, mental retardation, and reduced lifespan (including embryonic lethality) [118]. Knowledge of cholesterol metabolism has proven crucial to both the early diagnosis and treatment of SLOS.

A number of diagnostic approaches have been used to identify affected fetuses, including analysis of synthetic cholesterol intermediates. In 2005, Chevy et al. [119] argued against the utility of molecular diagnostic testing and instead advocated for routine GC-MS profiling of amniotic fluid (AF) in the diagnoses of developmental abnormalities attributed to defects in sterol intermediate synthesis. Clinical indications for AF sampling included fetal growth retardation, external male genitalia defect, and limb defects. Using GC-MS, these investigators were able to detect significant differences in AF levels of cholesterol, 7-dehydrocholesterol, and 8-dehydrocholesterol between normal and SLOS-affected infants. Of these sterol intermediates, these investigators concluded that 8-dehydrocholesterol level, given its long-term stability, was the best marker for SLOS. Results from this larger clinical population confirmed previous results by Abuelo et al. [120] and Tint et al. [121]. In addition, a urinalysis can also be performed as early as 12 weeks to diagnose SLOS [122].

Because the defect in DHCR7 leads to low plasma cholesterol levels and elevated 7- and 8-dehydrocholesterol levels, current treatment standards include dietary cholesterol supplementation with statin therapy [123]. There are also currently studies examining the benefits of antioxidants in the treatment of SLOS (E. Elias, personal communication).

CONCLUSIONS

Based on current evidence, it is clear that certain genetic polymorphisms leading to functional alterations of proteins involved in cholesterol uptake, mobilization, and de novo cholesterol synthesis can significantly impact human fertility. With the advent of personalized medicine, the identification and functional importance of polymorphisms and other genomic alterations related to reproductive function in men and women remains an important area of research. It is expected and anticipated that in the coming years, cholesterol-related diagnostic studies and therapeutic interventions will be refined based on the principles of personalized medicine.

REFERENCES

1. Hull MG, Glazener CM, Kelly NJ, Conway DI, Foster PA, Hinton RA, Coulson C, Lambert PA, Watt EM, Desai KM. Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed) 1985; 291:1693–1697.
2. Menken J, Larsen U. Estimating the incidence and prevalence and analyzing the correlates of infertility and sterility. Ann N Y Acad Sci 1994; 709:249–265.
3. Practice Committee of American Society for Reproductive M. Definitions of infertility and recurrent pregnancy loss: a committee opinion. Fertil Steril 2013; 99:63.
4. Thoma ME, McLain AC, Louis JF, King RB, Trumble AC, Sundaram R, Buck Louis GM. Prevalence of infertility in the United States as estimated by the current duration approach and a traditional constructed approach. Fertil Steril 2013; 99:1324–1331.e1.
5. Causes of Infertility [Internet]. Birmingham, AL: American Society of Reproductive Medicine. http://www.asrm.org/Infographic_Causes_of_Infertility/. Accessed April 2014.
6. Miller WL, Auchus RJ. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. Endocr Rev 2011; 32:81–151.
7. Rosenwaks Z, Adashi EY. Introduction: Fertility in the face of genetically determined steroidogenic dysfunction. Fertil Steril 2014; 101:299–300.
8. Marsh CA, Auchus RJ. Fertility in patients with genetic deficiencies of cytochrome P450c17 (CYP17A1): combined 17-hydroxylase/17, 20-lyase deficiency and isolated 17, 20-lyase deficiency. Fertil Steril 2014; 101:317–322.
9. Kang HJ, Imperato-McGinley J, Zhu YS, Rosenwaks Z. The effect of 5alpha-reductase-2 deficiency on human fertility. Fertil Steril 2014; 101: 310–316.
10. Bulun SE. Aromatase and estrogen receptor alpha deficiency. Fertil Steril 2014; 101:323–329.
11. Reichman DE, White PC, New MI, Rosenwaks Z. Fertility in patients with congenital adrenal hyperplasia. Fertil Steril 2014; 101:301–309.
12. Gwynne JT, Strauss JF III. The role of lipoproteins in steroidogenesis and cholesterol metabolism in steroidogenic glands. Endocr Rev 1982; 3: 299–329.
13. Stocco DM, Clark BJ. Regulation of the acute production of steroids in steroidogenic cells. Endocr Rev 1996; 17:221–244.
14. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science 1986; 232:34–47.

15. Brown MS, Kovanen PT, Goldstein JL. Receptor-mediated uptake of lipoprotein-cholesterol and its utilization for steroid synthesis in the adrenal cortex. Recent Prog Horm Res 1979; 35:215–257.
16. Goldstein JL, Brown MS. The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem 1977; 46:897–930.
17. Brown MS, Goldstein JL. Receptor-mediated control of cholesterol metabolism. Science 1976; 191:150–154.
18. 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 U S A 1997; 94: 12610–12615.
19. Thuahnai ST, Lund-Katz S, Anantharamaiah GM, Williams DL, Phillips MC. A quantitative analysis of apolipoprotein binding to SR-BI: multiple binding sites for lipid-free and lipid-associated apolipoproteins. J Lipid Res 2003; 44:1132–1142.
20. 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.
21. Kraemer FB, Shen WJ. Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis. J Lipid Res 2002; 43:1585–1594.
22. Bisgaier CL, Chanderbhan R, Hinds RW, Vahouny GV. Adrenal cholesterol esters as substrate source for steroidogenesis. J Steroid Biochem 1985; 23:967–974.
23. Andersen JM, Dietschy JM. Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat. J Biol Chem 1978; 253:9024–9032.
24. Spady DK, Dietschy JM. Rates of cholesterol synthesis and low-density lipoprotein uptake in the adrenal glands of the rat, hamster and rabbit in vivo. Biochim Biophys Acta 1985; 836:167–175.
25. Spady DK, Dietschy JM. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. J Lipid Res 1983; 24:303–315.
26. Espenshade PJ, Hughes AL. Regulation of sterol synthesis in eukaryotes. Annu Rev Genet 2007; 41:401–427.
27. Brown MS, Goldstein JL. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 1997; 89:331–340.
28. Stein JH, Rosenson RS. Lipoprotein Lp(a) excess and coronary heart disease. Arch Intern Med 1997; 157:1170–1176.
29. Lloyd JK. Lipoprotein deficiency disorders. Clinics Endocrinol Metab 1973; 2:127–147.
30. Borkowski AJ, Levin S, Delcroix C, Mahler A, Verhas V. Blood cholesterol and hydrocortisone production in man: quantitative aspects of the utilization of circulating cholesterol by the adrenals at rest and under adrenocorticotropin stimulation. J Clin Invest 1967; 46:797–811.
31. Bolte E, Coudert S, Lefebvre Y. Steroid production from plasma cholesterol. II. In vivo conversion of plasma cholesterol to ovarian progesterone and adrenal C19 and C21 steroids in humans.J Clin Endocrinol Metab 1974; 38:394–400.
32. Hellig H, Gattereau D, Lefebvre Y, Bolte E. Steroid production from plasma cholesterol. I. Conversion of plasma cholesterol to placental progesterone in humans. J Clin Endocrinol Metab 1970; 30:624–631.
33. Mahley RW, Rall SC Jr. Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000; 1:507–537.
34. Zhang T, Dai P, Cheng D, Zhang L, Chen Z, Meng X, Zhang F, Han X, Liu J, Pan J, Yang G, Zhang C. Obesity occurring in apolipoprotein E-knockout mice has mild effects on fertility. Reproduction 2014; 147: 141–151.
35. Utermann G, Hees M, Steinmetz A. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinaemia in man. Nature 1977; 269: 604–607.
36. Wenham PR, Price WH, Blandell G. Apolipoprotein E genotyping by one-stage PCR. Lancet 1991; 337:1158–1159.
37. Hatters DM, Peters-Libeu CA, Weisgraber KH. Apolipoprotein E structure: insights into function. Trends Biochem Sci 2006; 31:445–454.
38. Corbo RM, Scacchi R, Cresta M. Differential reproductive efficiency associated with common apolipoprotein e alleles in postreproductive-aged subjects. Fertil Steril 2004; 81:104–107.
39. Corbo RM, Ulizzi L, Piombo L, Scacchi R. Study on a possible effect of four longevity candidate genes (ACE, PON1, PPAR-gamma, and APOE) on human fertility. Biogerontology 2008; 9:317–323.
40. Corbo RM, Ulizzi L, Scacchi R, Martinez-Labarga C, De Stefano GF. Apolipoprotein E polymorphism and fertility: a study in pre-industrial populations. Mol Hum Reprod 2004; 10:617–620.
41. Gerdes LU, Gerdes C, Hansen PS, Klausen IC, Faergeman O. Are men carrying the apolipoprotein epsilon 4- or epsilon 2 allele less fertile than epsilon 3 epsilon 3 genotypes? Hum Genet 1996; 98:239–242.
42. Setarehbadi R, Vatannejad A, Vaisi-Raygani A, Amiri I, Esfahani M, Fattahi A, Tavilani H. Apolipoprotein E genotypes of fertile and infertile men. Syst Biol Reprod Med 2012; 58:263–267.
43. Brannian JD, Stouffer RL. Native and modified (acetylated) low density lipoprotein-supported steroidogenesis by macaque granulosa cells collected before and after the ovulatory stimulus: correlation with fluorescent lipoprotein uptake. Endocrinology 1993; 132:591–597.
44. Brannian JD, Shiigi SM, Stouffer RL. Gonadotropin surge increases fluorescent-tagged low-density lipoprotein uptake by macaque granulosa cells from preovulatory follicles. Biol Reprod 1992; 47:355–360.
45. Parinaud J, Perret B, Ribbes H, Chap H, Pontonnier G, Douste-Blazy L. High density lipoprotein and low density lipoprotein utilization by human granulosa cells for progesterone synthesis in serum-free culture: respective contributions of free and esterified cholesterol. J Clin Endocrinol Metab 1987; 64:409–417.
46. Volpe A, Coukos G, Uccelli E, Droghini F, Adamo R, Artini PG. Follicular fluid lipoproteins in preovulatory period and their relationship with follicular maturation and progesterone production by human granulosa-luteal cells in vivo and in vitro. J Endocrinol Invest 1991; 14:737–742.
47. Carr BR, MacDonald PC, Simpson ER. The role of lipoproteins in the regulation of progesterone secretion by the human corpus luteum. Fertil Steril 1982; 38:303–311.
48. Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest 1993; 92:883–893.
49. 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.
50. Trigatti B, Rayburn H, Vinals M, Braun A, Miettinen H, Penman M, Hertz M, Schrenzel M, Amigo L, Rigotti A, Krieger M. Influence of the high density lipoprotein receptor SR-BI on reproductive and cardiovascular pathophysiology. Proc Natl Acad Sci U S A 1999; 96:9322–9327.
51. Rinninger F, Heine M, Singaraja R, Hayden M, Brundert M, Ramakrishnan R, Heeren J. High Density Lipoprotein Metabolism in Low Density Lipoprotein Receptor-deficient Mice. J Lipid Res 2014; 55: 1914–1924.
52. Murao K, Terpstra V, Green SR, Kondratenko N, Steinberg D, Quehenberger O. Characterization of CLA-1, a human homologue of rodent scavenger receptor BI, as a receptor for high density lipoprotein and apoptotic thymocytes. J Biol Chem 1997; 272:17551–17557.
53. Rigotti A, Acton SL, Krieger M. The class B scavenger receptors SR-BI and CD36 are receptors for anionic phospholipids. J Biol Chem 1995; 270:16221–16224.
54. Calvo D, Gomez-Coronado D, Lasuncion MA, Vega MA. CLA-1 is an 85-kD plasma membrane glycoprotein that acts as a high-affinity receptor for both native (HDL, LDL, and VLDL) and modified (OxLDL and AcLDL) lipoproteins. Arterioscler Thromb Vasc Biol 1997; 17: 2341–2349.
55. 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.
56. Acton SL, Scherer PE, Lodish HF, Krieger M. Expression cloning of SR-BI, a CD36-related class B scavenger receptor. J Biol Chem 1994; 269: 21003–21009.
57. Gwynne JT, Hess B. The role of high density lipoproteins in rat adrenal cholesterol metabolism and steroidogenesis. J Biol Chem 1980; 255: 10875–10883.
58. Glass C, Pittman RC, Weinstein DB, Steinberg D. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. Proc Natl Acad Sci U S A 1983; 80:5435–5439.
59. Pittman RC, Knecht TP, Rosenbaum MS, Taylor CA, Jr. A non-endocytotic mechanism for the selective uptake of high density lipoprotein-associated cholesterol esters. J Biol Chem 1987; 262: 2443–2450.
60. Azhar S, Tsai L, Reaven E. Uptake and utilization of lipoprotein cholesteryl esters by rat granulosa cells. Biochim Biophys Acta 1990; 1047:148–160.
61. Reaven E, Chen YD, Spicher M, Azhar S. Morphological evidence that high density lipoproteins are not internalized by steroid-producing cells during in situ organ perfusion. J Clin Invest 1984; 74:1384–1397.
62. 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.
63. Reaven E, Tsai L, Azhar S. Intracellular events in the ''selective'' transport of lipoprotein-derived cholesteryl esters. J Biol Chem 1996; 271:16208–16217.
64. Jian B, de la Llera-Moya M, Ji Y, Wang N, Phillips MC, Swaney JB, Tall AR, Rothblat GH. Scavenger receptor class B type I as a mediator of cellular cholesterol efflux to lipoproteins and phospholipid acceptors. J Biol Chem 1998; 273:5599–5606.
65. de La Llera-Moya M, Connelly MA, Drazul D, Klein SM, Favari E, Yancey PG, Williams DL, Rothblat GH. Scavenger receptor class B type I affects cholesterol homeostasis by magnifying cholesterol flux between cells and HDL. J Lipid Res 2001; 42:1969–1978.
66. Azhar S, Reaven E. Scavenger receptor class BI and selective cholesteryl ester uptake: partners in the regulation of steroidogenesis. Mol Cell Endocrinol 2002; 195:1–26.
67. Li X, Peegel H, Menon KM. In situ hybridization of high density lipoprotein (scavenger, type 1) receptor messenger ribonucleic acid (mRNA) during folliculogenesis and luteinization: evidence for mRNA expression and induction by human chorionic gonadotropin specifically in cell types that use cholesterol for steroidogenesis. Endocrinology 1998; 139:3043–3049.
68. Rigotti A, Edelman ER, Seifert P, Iqbal SN, DeMattos RB, Temel RE, Krieger M, Williams DL. Regulation by adrenocorticotropic hormone of the in vivo expression of scavenger receptor class B type I (SR-BI), a high density lipoprotein receptor, in steroidogenic cells of the murine adrenal gland. J Biol Chem 1996; 271:33545–33549.
69. 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.
70. 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 1998; 39:1616–1628.
71. Jaspard B, Fournier N, Vieitez G, Atger V, Barbaras R, Vieu C, Manent J, Chap H, Perret B, Collet X. Structural and functional comparison of HDL from homologous human plasma and follicular fluid. A model for extravascular fluid. Arterioscler Thromb Vasc Biol 1997; 17:1605–1613.
72. Le Goff D. Follicular fluid lipoproteins in the mare: evaluation of HDL transfer from plasma to follicular fluid. Biochim Biophys Acta 1994; 1210:226–232.
73. Perret BP, Parinaud J, Ribbes H, Moatti JP, Pontonnier G, Chap H, Douste-Blazy L. Lipoprotein and phospholipid distribution in human follicular fluids. Fertil Steril 1985; 43:405–409.
74. Shalgi R, Kraicer P, Rimon A, Pinto M, Soferman N. Proteins of human follicular fluid: the blood-follicle barrier. Fertil Steril 1973; 24:429–434.
75. Simpson ER, Rochelle DB, Carr BR, MacDonald PC. Plasma lipoproteins in follicular fluid of human ovaries. J Clin Endocrinol Metab 1980; 51:1469–1471.
76. Bambauer R, Bambauer C, Lehmann B, Latza R, Schiel R. LDL-apheresis: technical and clinical aspects. Scientificworldjournal 2012; 2012:314283.
77. Mora S, Szklo M, Otvos JD, Greenland P, Psaty BM, Goff DC Jr, O'Leary DH, Saad MF, Tsai MY, Sharrett AR. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2007; 192:211–217.
78. Jimenez LM, Binelli M, Bertolin K, Pelletier RM, Murphy BD. Scavenger receptor-B1 and luteal function in mice. J Lipid Res 2010; 51:2362–2371.
79. 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.
80. Hirano K, Ikegami C, Tsujii K, Zhang Z, Matsuura F, Nakagawa-Toyama Y, Koseki M, Masuda D, Maruyama T, Shimomura I, Ueda Y, Yamashita S. Probucol enhances the expression of human hepatic scavenger receptor class B type I, possibly through a species-specific mechanism. Arterioscler Thromb Vasc Biol 2005; 25:2422–2427.
81. Miida T, Seino U, Miyazaki O, Hanyu O, Hirayama S, Saito T, Ishikawa Y, Akamatsu S, Nakano T, Nakajima K, Okazaki M, Okada M. Probucol markedly reduces HDL phospholipids and elevated prebeta1-HDL without delayed conversion into alpha-migrating HDL: putative role of angiopoietin-like protein 3 in probucol-induced HDL remodeling. Atherosclerosis 2008; 200:329–335.
82. Kolmakova A, Wang J, Brogan R, Chaffin C, Rodriguez A. Deficiency of scavenger receptor class B type I negatively affects progesterone secretion in human granulosa cells. Endocrinology 2010; 151: 5519–5527.
83. Christianson MS, Yates M. Scavenger receptor class B type 1 gene polymorphisms and female fertility. Curr Opin Endocrinol Diabetes Obes 2012; 19:115–120.
84. 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 high-density lipoprotein cholesterol levels in subjects with hyperalphalipoproteinemia. J Clin Endocrinol Metab 2009; 94: 1451–1457.
85. Yates M, Kolmakova A, Zhao Y, Rodriguez A. Clinical impact of scavenger receptor class B type I gene polymorphisms on human female fertility. Hum Reprod 2011; 26:1910–1916.
86. 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. Fertil Steril 2006; 85:1391–1397.
87. Brown MS, Goldstein JL, Krieger M, Ho YK, Anderson RG. Reversible accumulation of cholesteryl esters in macrophages incubated with acetylated lipoproteins. J Cell Biol 1979; 82:597–613.
88. Kraemer FB, Khor VK, Shen WJ, Azhar S. Cholesterol ester droplets and steroidogenesis. Mol Cell Endocrinol 2013; 371:15–19.
89. Yeaman SJ. Hormone-sensitive lipase–a multipurpose enzyme in lipid metabolism. Biochim Biophys Acta 1990; 1052:128–132.
90. Kraemer FB, Tavangar K, Hoffman AR. Developmental regulation of hormone-sensitive lipase mRNA in the rat: changes in steroidogenic tissues. J Lipid Res 1991; 32:1303–1310.
91. Park SY, Kim HJ, Wang S, Higashimori T, Dong J, Kim YJ, Cline G, Li H, Prentki M, Shulman GI, Mitchell GA, Kim JK. Hormone-sensitive lipase knockout mice have increased hepatic insulin sensitivity and are protected from short-term diet-induced insulin resistance in skeletal muscle and heart. Am J Physiol Endocrinol Metab 2005; 289:E30–39.
92. Kraemer FB, Shen WJ. Hormone-sensitive lipase knockouts. Nutr Metab (Lond) 2006; 3:12.
93. Osuga J, Ishibashi S, Oka T, Yagyu H, Tozawa R, Fujimoto A, Shionoiri F, Yahagi N, Kraemer FB, Tsutsumi O, Yamada N. Targeted disruption of hormone-sensitive lipase results in male sterility and adipocyte hypertrophy, but not in obesity. Proc Natl Acad Sci U S A 2000; 97: 787–792.
94. Hermo L, Chung S, Gregory M, Smith CE, Wang SP, El-Alfy M, Cyr DG, Mitchell GA, Trasler J. Alterations in the testis of hormone sensitive lipase-deficient mice is associated with decreased sperm counts, sperm motility, and fertility. Mol Reprod Dev 2008; 75:565–577.
95. Vallet-Erdtmann V, Tavernier G, Contreras JA, Mairal A, Rieu C, Touzalin AM, Holm C, Jegou B, Langin D. The testicular form of hormone-sensitive lipase HSLtes confers rescue of male infertility in HSL-deficient mice. J Biol Chem 2004; 279:42875–42880.
96. Garenc C, Perusse L, Chagnon YC, Rankinen T, Gagnon J, Borecki IB, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C. The hormone-sensitive lipase gene and body composition: the HERITAGE Family Study. Int J Obes Relat Metab Disord 2002; 26:220–227.
97. Shimada F, Makino H, Hashimoto N, Iwaoka H, Taira M, Nozaki O, Kanatsuka A, Holm C, Langin D, Saito Y. Detection of an amino acid polymorphism in hormone-sensitive lipase in Japanese subjects. Metabolism 1996; 45:862–864.
98. Magre J, Laurell H, Fizames C, Antoine PJ, Dib C, Vigouroux C, Bourut C, Capeau J, Weissenbach J, Langin D. Human hormone-sensitive lipase: genetic mapping, identification of a new dinucleotide repeat, and association with obesity and diabetes NIDDM. Diabetes 1998; 47: 284–286.
99. Talmud PJ, Palmen J, Luan J, Flavell D, Byrne CD, Waterworth DM, Wareham NJ. Variation in the promoter of the human hormone sensitive lipase gene shows gender specific effects on insulin and lipid levels: results from the Ely study. Biochim Biophys Acta 2001; 1537:239–244.
100. Talmud PJ, Palmen J, Walker M. Identification of genetic variation in the human hormone-sensitive lipase gene and 5′ sequences: homology of 5′ sequences with mouse promoter and identification of potential regulatory elements. Biochem Biophys Res Commun 1998; 252:661–668.
101. Vatannejad A, Khodadadi I, Amiri I, Vaisi-Raygani A, Ghorbani M, Tavilani H. Genetic variation of hormone sensitive lipase and male infertility. Syst Biol Reprod Med 2011; 57:288–291.
102. Porter FD, Herman GE. Malformation syndromes caused by disorders of cholesterol synthesis. J Lipid Res 2011; 52:6–34.
103. Ohashi M, Mizushima N, Kabeya Y, Yoshimori T. Localization of mammalian NAD(P)H steroid dehydrogenase-like protein on lipid droplets. J Biol Chem 2003; 278:36819–36829.
104. Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the

complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002; 109:1125–1131.
105. Song BL, Javitt NB, DeBose-Boyd RA. Insig-mediated degradation of HMG CoA reductase stimulated by lanosterol, an intermediate in the synthesis of cholesterol. Cell Metab 2005; 1:179–189.
106. Liu X, Li Y, Lu X, Wang L, Zhao Q, Yang W, Huang J, Cao J, Li H, Gu D. Interactions among genetic variants from SREBP2 activating-related pathway on risk of coronary heart disease in Chinese Han population. Atherosclerosis 2010; 208:421–426.
107. Steffen KM, Cooper ME, Shi M, Caprau D, Simhan HN, Dagle JM, Marazita ML, Murray JC. Maternal and fetal variation in genes of cholesterol metabolism is associated with preterm delivery. J Perinatol 2007; 27:672–680.
108. Knoblauch H, Bauerfeind A, Toliat MR, Becker C, Luganskaja T, Gunther UP, Rohde K, Schuster H, Junghans C, Luft FC, Nurnberg P, Reich JG. Haplotypes and SNPs in 13 lipid-relevant genes explain most of the genetic variance in high-density lipoprotein and low-density lipoprotein cholesterol. Hum Mol Genet 2004; 13:993–1004.
109. Rozman D, Seliskar M, Cotman M, Fink M. Pre-cholesterol precursors in gametogenesis. Mol Cell Endocrinol 2005; 234:47–56.
110. Lewinska M, Zelenko U, Merzel F, Golic Grdadolnik S, Murray JC, Rozman D. Polymorphisms of CYP51A1 from cholesterol synthesis: associations with birth weight and maternal lipid levels and impact on CYP51 protein structure. PLoS One 2013; 8:e82554.
111. Lange Y, Ory DS, Ye J, Lanier MH, Hsu FF, Steck TL. Effectors of rapid homeostatic responses of endoplasmic reticulum cholesterol and 3-hydroxy-3-methylglutaryl-CoA reductase. J Biol Chem 2008; 283: 1445–1455.
112. Zerenturk EJ, Sharpe LJ, Ikonen E, Brown AJ. Desmosterol and DHCR24: unexpected new directions for a terminal step in cholesterol synthesis. Prog Lipid Res 2013; 52:666–680.
113. FitzPatrick DR, Keeling JW, Evans MJ, Kan AE, Bell JE, Porteous ME, Mills K, Winter RM, Clayton PT. Clinical phenotype of desmosterolosis. Am J Med Genet 1998; 75:145–152.
114. Schaaf CP, Koster J, Katsonis P, Kratz L, Shchelochkov OA, Scaglia F, Kelley RI, Lichtarge O, Waterham HR, Shinawi M. Desmosterolosis-phenotypic and molecular characterization of a third case and review of the literature. Am J Med Genet A 2011; 155A:1597–1604.
115. McGrath KC, Li XH, Puranik R, Liong EC, Tan JT, Dy VM, DiBartolo BA, Barter PJ, Rye KA, Heather AK. Role of 3beta-hydroxysteroid-delta 24 reductase in mediating antiinflammatory effects of high-density lipoproteins in endothelial cells. Arterioscler Thromb Vasc Biol 2009; 29:877–882.
116. Lu X, Kambe F, Cao X, Kozaki Y, Kaji T, Ishii T, Seo H. 3beta-Hydroxysteroid-delta24 reductase is a hydrogen peroxide scavenger, protecting cells from oxidative stress-induced apoptosis. Endocrinology 2008; 149:3267–3273.
117. Greeve I, Hermans-Borgmeyer I, Brellinger C, Kasper D, Gomez-Isla T, Behl C, Levkau B, Nitsch RM. The human DIMINUTO/DWARF1 homolog seladin-1 confers resistance to Alzheimer's disease-associated neurodegeneration and oxidative stress. J Neurosci 2000; 20:7345–7352.
118. Tint GS, Irons M, Elias ER, Batta AK, Frieden R, Chen TS, Salen G. Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. N Engl J Med 1994; 330:107–113.
119. Chevy F, Humbert L, Wolf C. Sterol profiling of amniotic fluid: a routine method for the detection of distal cholesterol synthesis deficit. Prenat Diagn 2005; 25:1000–1006.
120. Abuelo DN, Tint GS, Kelley R, Batta AK, Shefer S, Salen G. Prenatal detection of the cholesterol biosynthetic defect in the Smith-Lemli-Opitz syndrome by the analysis of amniotic fluid sterols. Am J Med Genet 1995; 56:281–285.
121. Tint GS, Abuelo D, Till M, Cordier MP, Batta AK, Shefer S, Honda A, Honda M, Xu G, Irons M, Elias ER, Salen G. Fetal Smith-Lemli-Opitz syndrome can be detected accurately and reliably by measuring amniotic fluid dehydrocholesterols. Prenat Diagn 1998; 18:651–658.
122. Shackleton CH, Roitman E, Kratz L, Dehydro-oestriol Kelley R. and dehydropregnanetriol are candidate analytes for prenatal diagnosis of Smith-Lemli-Opitz syndrome. Prenat Diagn 2001; 21:207–212.
123. Svoboda MD, Christie JM, Eroglu Y, Freeman KA, Steiner RD. Treatment of Smith-Lemli-Opitz syndrome and other sterol disorders. Am J Med Genet C Semin Med Genet 2012; 160C:285–294.