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Loss of apolipoprotein E exacerbates the neonatal lethality of the Smith-Lemli-Opitz syndrome mouse

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Abstract

The Smith-Lemli-Opitz syndrome (SLOS) is caused by a genetic defect in cholesterol biosynthesis; mutations in the enzyme 3 β -hydroxysterol Δ 7 reductase (*Dhcr7*) lead to a failure of cholesterol (and desmosterol) synthesis, with an accumulation of precursor sterols, such as 7-dehydrocholesterol. Extensive genotype–phenotype analyses have indicated that there is considerable variation in the severity of the disease, much of which is not explained by defects in the *Dhcr7* gene alone. Factors ranging from variations in maternal–fetal cholesterol transfer during pregnancy, to other genetic factors have been proposed to account for this variability. Variations at the *APOE* locus affect plasma cholesterol levels in humans and this polymorphic gene has been found to be associated with cardiovascular as well as neurological disorders. This locus has recently been implicated in accounting for some of the variations in SLOS. To address whether maternal hypercholesterolemia can affect fetal outcome, we tested the ability of maternal hypercholesterolemia to rescue the neonatal lethality in a mouse model of SLOS. Maternal hypercholesterolemia, induced by ApoE or Ldl-r deficiency not only failed to ameliorate the postnatal lethality, it increased the prenatal mortality of *Dhcr7* deficient pups. Thus the murine data suggest that maternal loss of ApoE or Ldl-r function further exacerbates the neonatal lethality, suggesting they may play a role in maternal transfer of cholesterol to the embryo.

Keywords

Genetics; RSH syndrome; Sterols; Maternal–fetal transfer; Fetal development

Introduction

The Smith-Lemli-Opitz syndrome (SLOS), is an autosomal recessive disorder of sterol metabolism characterized by the loss of enzymatic activity of 3 β -hydroxysterol Δ 7 reductase (*Dhcr7*) [1–7]. This leads to a classical dysmorphism/malformation syndrome, with severity ranging from early embryonic loss to variable survival after birth. Developmental delay and mental retardation in survivors are almost invariant features, although a few exceptions have

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been described [1]. The incidence of SLOS is estimated to be between 1:20,000–80,000 live births, dependent upon geographic occurrence, with a carrier frequency for mutated *DHCR7* alleles of ~1.4% in the Caucasian population [8–10]. *DHCR7* catalyzes the reduction of the C7–C8 double bond of precursor sterols, a necessary step in the synthesis of cholesterol. As a result, in SLOS patients, there is a deficiency of cholesterol in tissues, with simultaneous accumulation of precursor sterols, mainly 7- and 8-dehydrocholesterols. A wide spectrum of mutations in *DHCR7* have now been reported to cause SLOS, with three mutations accounting for more than 70% of the mutant alleles [2–5,10]. Although SLOS was initially classified clinically into two groups, SLO type I and SLO type II, based upon clinical criteria and severity, current observations suggest that these are but two ends of a continuous spectrum of severity. In previous investigations, we and others have shown that severity of SLOS disease correlates with plasma cholesterol levels, but that this does not account for all of the observed variations in severity [5,10]. Additionally, it is not uncommon to note dissimilar phenotypes in affected siblings, suggesting that factors other than mutations in *DHCR7* affect phenotype. Factors that have the potential to ameliorate or exacerbate severity include those that might increase the maternal–fetal transfer of cholesterol, increase or decrease clearance of ‘toxic’ precursor sterols, increase or decrease plasma sterols, as well as factors that modify the response to sterol deficiency. While environmental and acquired factors are more difficult to quantify, the genetic contribution to the above processes can be tested. The latter are comprised of the maternal genotype and the paternally contributed fetal genotype. A recent study of a large cohort of SLOS pedigrees suggested that a major genetic factor might be variations at the APOE locus, encoding apolipoprotein E [11].

Human apolipoprotein E (APOE) plays a vital role in lipoprotein metabolism [12]. It is one of the genetic determinants of plasma total cholesterol and LDL cholesterol. APOE has been shown to play a role in lipid metabolic disorders causing vascular diseases, atherosclerosis, coronary artery disease, and myocardial infarction [13], and it seems to play a role in pre-disposition to Alzheimer's disease [14]. The *APOE* gene is polymorphic in nature, characterized by presence of three common alleles $\epsilon 2$ (APOE2 protein), $\epsilon 3$ (APOE3), and $\epsilon 4$ (APOE4) and several much rarer alleles ($\epsilon 1$, $\epsilon 5$, etc) [15]. The role of *APOE* variations in determining plasma levels and neurological function has been extensively studied [12]. Polymorphisms in *APOE* can also alter the response to dietary modifications [16] and treatment with lipid lowering drugs [17]. It has been well established that the isoforms of APOE help determine the lipid and lipoprotein content of plasma; the $\epsilon 4$ allele is associated with higher and the $\epsilon 2$ allele with lower LDL-cholesterol and apolipoprotein B (APOB) levels [18] while higher plasma triglycerides are more commonly associated with the $\epsilon 4$ and $\epsilon 2$ alleles compared to $\epsilon 3$. Interestingly, the maternal *APOE* locus influences lipoprotein levels in newborns and vice versa; the $\epsilon 2$ isoform in newborns is associated with elevated maternal LDL-C and APOB levels and the same isoform in the mother is associated with a decreased in LDL-C and APOB and increased HDL-C levels in newborn cord blood [19,20]. Transfer of maternal cholesterol to embryos via the placenta and yolk sac has been documented in murine models [21]. In previous experiments, feeding a diet rich in cholesterol to pregnant dams carrying *Dhcr7* null embryos failed to ameliorate the lethality (Tint and Yu, unpublished observations). Since diet does not induce a significant hypercholesterolemia in these mice, we isolated a knockout mouse model of SLOS, on a maternal background of ApoE or Ldl-receptor (Ldl-r) deficiency [22]. Ldl-r or ApoE deficiency leads to hypercholesterolemia in mice on a normal chow diet, which can be further exacerbated by a high (2%) cholesterol diet [23–26]. Dams (*Dhcr7*^{+/-}) that were, in addition, either *ApoE*^{-/-} or *Ldl-r*^{-/-} (and thus had high plasma cholesterol levels during pregnancy) were bred with heterozygous males (*Dhcr7*^{+/-}). Their *Dhcr7*^{+/-} progeny (all of which are hemi-deficient for ApoE or Ldl-r) were examined for severity to test the hypothesis that maternal hypercholesterolemia *per se* may be able to ameliorate the cholesterol deficiency in the SLOS pups.

Materials and methods

Breeding of Dhcr7 KO mice in ApoE or Ldl-r KO background

The VA Institutional Animal Care and Use Committee (IACUC) approved all animal research protocols. C57Bl/6J *ApoE*^{-/-} or *Ldl-r*^{-/-} KO mice were obtained from the Jackson Laboratory, ME and *Dhcr7*^{+/-}, described earlier, had been backcrossed onto the C57Bl/6J strain for *N* > 14 generations before intercrossing with these KO lines. Animals were maintained on rodent chow diet. A limited number of *ApoE*^{-/-} *Dhcr7*^{+/-} and *Ldl-r*^{-/-} *Dhcr7*^{+/-} female mice were fed with 2% cholesterol diet starting at an age of 8 weeks and specifically 1 week before the planned copulation (see below). Breeding was carried out with *Dhcr7*^{+/-} mice and *ApoE*^{-/-} or *Ldl-r*^{-/-} KO mice to generate *ApoE*^{+/-}, *Dhcr7*^{+/-} or *Ldl-r*^{+/-}, *Dhcr7*^{+/-} mice. Backcrossing these animals with the *ApoE*^{-/-} or *Ldl-r*^{-/-} KO mice generated female mice with either *ApoE*^{-/-}, *Dhcr7*^{+/-} or *Ldl-r*^{-/-}, *Dhcr7*^{+/-} genotypes which were used as dams. These dams were mated with male *Dhcr7*^{+/-} to generate pups that are heterozygous for either the *ApoE* or the *Ldl-r* loci, but should result in all possible genotypes at the *Dhcr7* locus.

Characterization of pups

Genomic DNA was isolated from tails and PCR for *Dhcr7* status was carried out as described previously [22]. Oligonucleotides 5'-CTTACTCTACACAGGATGCCTAGCC-3' and 5'-TTCCCAGAAGTTGAGAAGCTGCGG-3' were used to genotype the *ApoE* status; a 467 bp product was obtained for wild type alleles and a 1.3 kb product for the disrupted allele. Oligonucleotides *ldlr-f* 5'-ACCCCAAGACGTGCTCCCAGGATG-3', *ldlr-r* 5'-CGCAGTGCTCCTCATCTGACTTGTC-3' and Neo 5'-AGGTGAGATGACAGGAGATC-3' were used to set up two separate PCRs (*ldlr-f* with *ldlr-r* and *ldlr-f* with Neo) to genotype for the *Ldl-r* status.

Characterization of embryos

Between days 10 and 11 after copulation (E10–11) the pregnant female mice were sacrificed and uteri harvested. Under a dissection microscope (Olympus SZX 12, Olympus America Inc., Center Valley, PA) the uterine horns were carefully opened, all fetal sacs counted and embryos with yolk sac and placenta were extracted one by one. After several rinses in PBS to eliminate possible contamination with maternal DNA, genomic DNA was isolated directly from the embryos for genotyping.

Measurement of triglycerides and total cholesterol in female mice

Blood was obtained from non-fasting animals at 2.00 p.m. by puncturing the retrobulbar venous plexus and plasma collected for total cholesterol and triglyceride determinations. Blood was collected one day before mating day and 15 days after appearance of a copulatory plug. For mice on 2% cholesterol diet blood was collected one day before and 1 week after the start date of the 2% cholesterol diet. Cholesterol was measured using the Cholesterol CII kit from Wako Chemicals, USA, following manufacturer's protocol. In brief, 10 µl of plasma was mixed with the color reagent and incubated for 5 min at 37 °C. The developed color was read at 505 nm in a colorimeter and quantitated against a standard curve. Triglyceride was measured using Infinity Triglycerides Reagent (Sigma, St. Louis, MO, USA) in a similar fashion.

Measurement of sterols in brains and livers of newborn mice

Newborn mice were observed closely for the first 12 h after birth and up to 24 h for survivors. Carcasses of dead newborn mice were immediately separated from their littermates and processed as described below. All living newborn pups were euthanatized by decapitation after 24 h. Brain and liver were dissected, flash frozen in liquid nitrogen and stored at -80 °C. Tissue sterols were measured as described previously [22].

Statistical analysis

For cholesterol and triglyceride values, comparisons among the study groups were evaluated by 1-way ANOVA, followed by Student's *t*-test. The expected Mendelian ratio at birth of the offspring from matings between *Dhcr7*^{+/-}, *Ldl-r*^{-/-} *Dhcr7*^{+/-} or *ApoE*^{-/-} *Dhcr7*^{+/-} females and *Dhcr7*^{+/-} males was compared to the actual ratio using the chi square test. The same test was performed for E10–11 embryos of *Ldl-r*^{-/-} *Dhcr7*^{+/-} and *ApoE*^{-/-} *Dhcr7*^{+/-} females. Statistical significance was defined as *P* ≤ 0.05.

Results

Plasma lipid profiles in *Dhcr7*^{+/-} deficient in *ApoE* or *Ldl-r* on rodent chow diet

Plasma total cholesterol and triglyceride levels in chowfed *ApoE*^{-/-} *Dhcr7*^{+/-} mice and *Dhcr7*^{+/-} mice are summarized in Table 1. No differences in triglyceride levels were noted. Total cholesterol was about 7-fold higher in *ApoE*^{-/-} *Dhcr7*^{+/-} mice compared to *Dhcr7*^{+/-} mice, as would be expected (*P* < 0.001).

Total cholesterol content was about 2.5-fold higher in *Ldl-r*^{-/-} *Dhcr7*^{+/-} mice compared to *Dhcr7*^{+/-} mice (*P* < 0.001). The *Ldl-r*^{+/-} *Dhcr7*^{+/-} showed an intermediate cholesterol value of 160 ± 32 mg/dL. No differences in triglyceride levels were noted (Table 1).

Total cholesterol before and during the pregnancy on rodent chow diet and on 2% cholesterol diet

No significant differences were found between plasma total cholesterol before and during the pregnancy (day 15 after copulation) in both *ApoE*^{-/-} *Dhcr7*^{+/-} (437 ± 105 mg/dL and 430 ± 107 mg/dL) and *Ldl-r*^{-/-} *Dhcr7*^{+/-} (191 ± 74 mg/dL and 236 ± 107 mg/dL) female mice on rodent chow diet. On 2% cholesterol diet total cholesterol values increased dramatically up to 1341 ± 250 mg/dL for *ApoE*^{-/-} *Dhcr7*^{+/-} and 1432 ± 322 mg/dL for *Ldl-r*^{-/-} *Dhcr7*^{+/-} (Fig. 1, *P* < 0.001). Cholesterol levels on 2% cholesterol diet were measured on not yet pregnant female mice.

Effect of a 2% cholesterol diet on fertility of *ApoE*^{-/-} *Dhcr7*^{+/-} or *Ldl-r*^{-/-} *Dhcr7*^{+/-} female mice

Table 2 shows a summary of the Effects of diet on the fertility of the *ApoE*^{-/-} *Dhcr7*^{+/-} and *Ldl-r*^{-/-} *Dhcr7*^{+/-} female mice. On a 2% cholesterol diet, the fertility of our mice was reduced and furthermore after 3 weeks female from both strains generally looked sick. Under these conditions, mating of *ApoE*^{-/-} *Dhcr7*^{+/-} was possible only two times per animal (they either died or were suffering and were euthanized). Thus, long-term experiments on this diet were not possible.

Lethality of the newborn pups from *ApoE*^{-/-} *Dhcr7*^{+/-} (female) and *Dhcr7*^{+/-} (male) mice, and *Ldl-r*^{-/-} *Dhcr7*^{+/-} (female) and *Dhcr7*^{+/-} (male) mice

We evaluated genotypes and lethality of pups of dams with a 'high plasma cholesterol' background (as shown above) on either rodent chow diet or 2% cholesterol diet. Neonatal lethality was defined as death within 24 h and usually within 12 h. We noted several pups of different *Dhcr7* genotypes on *ApoE* or *Ldl-r* heterozygous background. Thus it was possible to obtain all three genotypes in the progeny of hypercholesterolemic *Dhcr7*^{+/-} dams (that were either *ApoE* or *Ldl-r* null), mated with *Dhcr7*^{+/-} males. The results are summarized in Table 3. Independent of diet, all the *Dhcr7* homozygous knockout pups that were born died within 24 h. Interestingly on a 2% cholesterol diet, the overall pup lethality increased, reaching up to 57% in *ApoE*^{+/-} *Dhcr7*^{+/-} pups and 21% in *Ldl-r*^{+/-} *Dhcr7*^{+/-} pups, neither of which was expected. In crosses from *Dhcr7*^{+/-} dams with *Dhcr7*^{+/-} males, the frequencies of the genotypes of the pups was very close to the expected Mendelian ratios (Table 3, right columns). When

the ApoE or *Ldl-r* null dams were placed on a 2% cholesterol diet, we noted that not only were the litter sizes very small and fertility reduced suggesting a 'lethal' effect of the 2% cholesterol diet, though the reasons for this do not seem to be clear. Cholic acid was not present in this diet mix. On a rodent chow diet, *Dhcr7* wild type or heterozygotes survived apparently with no problems. This suggests that a 2% cholesterol diet is toxic to these mice and such a diet may not be a useful way to increase maternal plasma cholesterol in mice. Toxicity of the 2% cholesterol diet on *Dhcr7*^{+/-} dams (on a C57Bl strain background) was not studied, though this diet does not ameliorate the *Dhcr7*^{-/-} newborn pup lethality (Yu, unpublished observations).

Prenatal mortality of ApoE^{-/-} Dhcr7^{+/-} or Ldl-r^{+/-} Dhcr7^{-/-} mice

Dhcr7 knockout homozygous pups born from ApoE^{-/-} *Dhcr7*^{+/-} female mated with *Dhcr7*^{+/-} male mice deviated from the expected Mendelian ratio, showing increased prenatal mortality (Table 3). Although the χ^2 test showed no significant difference on a 2% cholesterol diet (presumably because of the small sample size), the results were significant when considering either chow diet alone or both diets together.

The effect of the loss of Ldl-r in the dams was milder and, although no statistical differences were noted (*Dhcr7*^{+/+} 27%, *Dhcr7*^{+/-} 58%, *Dhcr7*^{-/-} 15%, $P > 0.5$, Table 3), there was a trend toward early fetal demise.

Embryonic loss of ApoE^{+/-} Dhcr7^{-/-} genotypes

To determine at what stage the early fetal loss was occurring, dams at E10–11 were sacrificed and all fetuses genotyped. As shown in Table 4, at E10–11 there was a tendency (but not significant, $P \sim 0.1$) towards a decrease in the expected *Dhcr7*^{-/-} embryos, although only limited numbers of pregnancies were observed. Based upon this, lethality was estimated to occur in the second trimester, between E9–13 in these mice.

Sterol analyses of liver and brain tissues in newborn pups

Livers of *Dhcr7*^{-/-} animals on ApoE^{+/-} or *Ldl-r*^{+/-} background were clearly deficient in cholesterol content and showed a significant increase in the precursors 7- and 8-DHC (Table 5). Similar profile trends were detected in brain of *Dhcr7*^{-/-} animals on ApoE^{+/-} background. Our results are similar to previous publications [22,27], where data regarding sterol profiles of *Dhcr7* heterozygous animals, as well as sterols during development and in newborn pups are available.

Discussion

Apolipoprotein E exerts its role on the lipoprotein metabolic pathways through interactions with different classes of lipoprotein receptors and affects lipoprotein clearance as well as intestinal cholesterol absorption [12,28]. Polymorphisms at the *APOE* locus influence these parameters. APOE2 carriers have lower levels of total cholesterol, LDL cholesterol and apolipoprotein B levels than APOE3 homozygotes. In contrast, APOE4 is associated with higher levels of total, LDL cholesterol and apolipoprotein B levels. APOE has two functional domains that can be generated by thrombin cleavage; the N-terminal fragment contains the receptor-binding domain and the C terminal fragment contains the lipoprotein-binding domain. Although the polymorphic residues 112 and 158 lie in the N-terminal domain, interactions between different residues in the N-terminal domain alter the ability of the lipid-binding domain to interact and bind to various phospholipids [29].

In SLOS, there is a deficiency of cholesterol synthesis as well as a large accumulation of precursor sterols in the developing embryo. Genotype–phenotype correlations show that a wide-spectrum of mutations in the *DHCR7* gene can cause SLOS, but these mutations may

account for only an estimated 50% of the severity of the disease [10,30]. Within a single family, the severity scores of two affected siblings can be different [10]. Thus either environmental and/or other genetic factors may play a modulating role. Although mammalian embryos from the zygote stage can synthesize cholesterol, there is strong evidence in animal models to indicate that maternally derived cholesterol can be detected in the developing embryo [21]. Thus, under conditions of fetal deficiency, one modulating factor could be maternal delivery of this cholesterol that could ameliorate this deficiency. Maternal cholesterol in humans rises during pregnancy and cholesterol levels are affected by a number of factors, environmental and genetic. One candidate is therefore the *APOE* locus, either by its effects directly in the developing fetus, on fetal lipoprotein metabolism, or by affecting maternal cholesterol levels and materno-fetal lipoprotein metabolism [19,20]. A recent study has implicated maternal *APOE* variations as determinants of severity in SLOS [11]. Maternal, but not fetal, *APOE2* allele genotype was associated with a poorer clinical severity score. However, in one case, where the mother was homozygous for *APOE2*, severity score of the patient was lower, suggesting this effect is not dose dependent [11]. Additionally, given the significant possible errors that can occur when the sample size used in genetic polymorphism association studies is low [31,32], the role of maternal *APOE* status affecting clinical severity in SLOS should be regarded as probable, but not proven [32].

We tested the possibility that elevating maternal cholesterol could still play a role in altering fetal outcome in a mouse model of SLOS. Maternal plasma sterol levels, during pregnancy carrying affected children in SLOS have not been reported. Using two different genetic manipulations to induce hypercholesterolemia in the pregnant mouse carrying *Dhcr7* deficient fetuses still failed to ameliorate the neonatal lethality of *Dhcr7* deficiency. Mouse maternal hypercholesterolemia was induced by *ApoE* deficiency or by *Ldl-r* deficiency. In both cases, we also fed the pregnant dams on cholesterol-fortified diets to ensure sustained and comparable mean plasma cholesterol levels. Our results showed that loss of maternal *ApoE* or *Ldl-r* function, despite leading to significant plasma hypercholesterolemia, exacerbated the SLOS phenotype. An early embryonic loss of *Dhcr7*^{-/-} was noted. Our interpretation of these data is that both *ApoE* and *Ldl-r* are important in maternal transfer of cholesterol during the early stages of embryonic development, a step that is made critical, when embryos cannot synthesize their own cholesterol, as is the case with post-squalene genetic disorders [7]. Although it is known that secretion of lipoproteins by the murine yolk sac is important for early embryonic development (as indicated by the lethality of *Mtp* or *ApoB* knockout mice [33-35]), loss of *ApoE* or *Ldl-r* does not seem to affect embryonic viability. Our data suggest that these steps may be important, when cholesterol synthesis in the embryo is rate-limiting. There are some caveats to our conclusions. To ensure that the affected fetuses did not have their lipoprotein metabolism altered by the maternal genetic manipulations, all the fetuses were heterozygous with respect to the *ApoE* or *Ldl-r* alleles by virtue of inheriting a functional allele from the paternal side. Mice heterozygous for *ApoE* null alleles seem to be indistinguishable from wild type mice when fed a chow diet and their lipoprotein kinetics seem to be similar [26,36]. Even mice with hypomorphic *ApoE* alleles seem to be similar to wild type mice, unless a severe hypercholesterolemic diet is imposed [37]. However, a formal study examining embryonic development in pups heterozygous for either *ApoE* or *Ldl-r* null alleles has not been reported. Based upon current knowledge, we do not think that heterozygosity for *ApoE* or *Ldl-r* null alleles of fetuses affected embryonic development.

Another caveat is that the mouse may not be the optimal model to test these hypotheses (of maternal hypercholesterolemia improving SLOS fetal outcomes), as the predominant lipoprotein in the mouse is the high-density lipoprotein. Although cholesterol elevations in the *ApoE*^{-/-} and *Ldl-r*^{-/-} mice are mainly in the VLDL and LDL range, HDL cholesterol is also elevated, though these elevations are very modest. Additionally, there may be important differences between rodent maternal-fetal transfer of cholesterol and that in humans. Loss of

apolipo-protein B or MTP (as in human disorders of Hypobetalipoproteinemia, MIM 605019 and Abetalipoproteinemia, MIM 200100, respectively) does not seem to lead to fetal demise in humans (or cause dysmorphology), whereas loss of these genes in mice result in embryonic lethality [33,38]. Thus, the transfer of lipoproteins between the yolk sac and the developing fetus is critical in rodents, but apparently not so in humans. On the other hand, there is evidence in humans that fetal genotypes can affect maternal lipid levels [20]. This would suggest some biological crosstalk between these two entities. Our data are compatible with the role of lipoproteins in maternal–fetal cholesterol transfer. However, this transfer may be quantitatively more important at the level of cellular transfer between the placental unit and the fetus, but not at the level of maternal circulation.

Finally, our mouse models reflect hypercholesterolemia, as a result of a *loss* of function (either ApoE or Ldl-r). It is possible that mouse models that have been humanized with the APOE variants (APOE2, APOE3 or APOE4) may provide a better model to study the effects of APOE variations, as these express the variant APOEs and thus have lipoprotein kinetics significantly different than null mice [39,40]. This experiment is currently limited by the fact that the *ApoE* locus is on the same chromosome as *Dhcr7* and to generate mice that are APOE2/*Dhcr7*^{+/-} would require a recombination event and this may take a significant amount of breeding resources to generate each of the APOE alleles.

In conclusion, raising maternal plasma cholesterol, either by ApoE or Ldl-r deficiency not only failed to ameliorate the neonatal lethality in a mouse model of SLOS, it exacerbated this phenotype. This latter observation suggest that cholesterol transfer from the mother to the fetus may involve more local factors, such as the placental cells and local delivery that involves ApoE and Ldl-r. If so, manipulations that lead to an increase in placental cholesterol synthesis and transfer may lead to greater fetal viability.

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Fig. 1. *ApoE*^{-/-} *Dhcr7*^{+/-} (A) or *Ldl-r*^{-/-} *Dhcr7*^{+/-} (B) female mice total cholesterol in plasma. Plasma was collected on rodent chow diet one day before mating date (black) and 15 days after copulation (white) and for mice on 2% cholesterol diet 1 week after starting a 2% cholesterol diet (gray) and before copulation. **P* < 0.001.

Table 1

Plasma lipid levels in adult animals

Genotype		Total cholesterol (mg/dL)	Triglycerides (mg/dL)
<i>ApoE</i> ^{+/+} <i>Dhcr7</i> ^{+/-}	(N = 10)	80.7 ± 12.1	59 ± 11
<i>ApoE</i> ^{-/-} <i>Dhcr7</i> ^{+/-}	(N = 10)	569 ± 85*	61 ± 22
<i>Ldl-r</i> ^{+/+} <i>Dhcr7</i> ^{+/-}	(N = 6)	119 ± 14	58 ± 11
<i>Ldl-r</i> ^{-/-} <i>Dhcr7</i> ^{+/-}	(N = 11)	307 ± 47*	54 ± 22

* $P < 0.001$.

Table 2Fertility rates of *ApoE*^{-/-}*Dhcr7*^{+/-} and *Ldl-r*^{-/-}*Dhcr7*^{+/-} placed on rodent chow diet or 2% cholesterol diet

	2% cholesterol diet	Rodent chow diet (%)
<i>ApoE</i> ^{-/-} <i>Dhcr7</i> ^{+/-} female		
Matings without litter	62.5%	45.5
Matings with litter	37.5%	54.5
<i>Ldl-r</i> ^{-/-} <i>Dhcr7</i> ^{+/-} female		
Matings without litter	61.5%	50
Matings with litter	38.5%	50

Dhcr7^{+/-} females deficient for ApoE or Ldl-r were mated for 3 days with *Dhcr7*^{+/-} males (*N* = 6). During the mating period two female mice were transferred in male cages.

Table 3

Viability of the pups grouped by parental genotypes and diet

Genotype	Pups from <i>ApoE</i> ^{+/-} <i>Dhcr7</i> ^{+/-} male		<i>ApoE</i> ^{+/-} <i>Dhcr7</i> ^{+/-} female and <i>ApoE</i> ^{+/-} <i>Dhcr7</i> ^{+/-}		Pups from <i>Ldlr</i> ^{+/-} <i>Dhcr7</i> ^{+/-} male		<i>Ldlr</i> ^{+/-} <i>Dhcr7</i> ^{+/-} female and <i>Ldlr</i> ^{+/-} <i>Dhcr7</i> ^{+/-}		Pups from <i>Dhcr7</i> ^{+/-} male	
	<i>ApoE</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>ApoE</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>ApoE</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>ApoE</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>Ldlr</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>Ldlr</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>Ldlr</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>Ldlr</i> ^{+/-} <i>Dhcr7</i> ^{+/-}	<i>Dhcr7</i> ^{+/-}	<i>Dhcr7</i> ^{+/-}
Rodent chow diet	24 *	68 *	9 *	19 *	41 *	9 *	28	59	28	28
Lethality	0%	1%	100%	0%	0%	100%	0%	0%	0%	100%
2% cholesterol diet	5	7	1	6	14	5				
Lethality	40%	57%	100%	16%	21%	100%				
Total Number	29 *	75 *	10 *	25	55	14	28	59	28	28
Lethality	7%	7%	100%	4%	5%	100%	0%	0%	0%	100%

* $\chi^2 = 9.8, P < 0.05$.

Table 4Dhcr7 genotype distributions of embryos at E10–11 in *ApoE*^{-/-}*Dhcr7*^{+/-} dams

Embryos	Observed no. embryos (%)	Expected no. of embryos (%)
+/+	11 (32)	10 (25)
+/-	19 (56)	20 (50)
-/-	4 (12)	10 (25)
Total	34 (100)	40 (100)

Table 5 Sterol concentrations in brain and liver from newborn *ApoE^{+/-}Dhcr7^{-/-}*, *ApoE^{+/-}Dhcr7^{+/-}*, *Ldl-r^{+/-}dhc-7^{-/-}* and *Ldl-r^{+/-}Dhcr7^{+/-}* pups

	Brain tissue (mg/g) (mean ± SD)		Liver tissue (mg/g) (mean ± SD)		Liver tissue (mg/g) (mean ± SD)	
	<i>Dhcr7^{+/-}ApoE^{+/-}</i>	<i>Dhcr7^{-/-}ApoE^{+/-}</i>	<i>Dhcr7^{+/-}ApoE^{+/-}</i>	<i>Dhcr7^{-/-}ApoE^{+/-}</i>	<i>Dhcr7^{+/-}Ldl-r^{+/-}</i>	<i>Dhcr7^{-/-}Ldl-r^{+/-}</i>
<i>N</i>	6	3	6	3	6	3
Cholesterol	3.53 ± 0.27	0.80 ± 0.50 [*]	5.08 ± 3.44	0.74 ± 0.23 [*]	3.57 ± 1.13	1.15 ± 0.43 [*]
7DHC	—	2.27 ± 0.95	—	1.66 ± 0.70	—	1.21 ± 0.73
8DHC	—	0.32 ± 0.13	—	0.29 ± 0.06	—	0.23 ± 0.10
Desmosterol	0.96 ± 0.05	—	—	—	—	—
Lathosterol	—	0.19 ± 0.17	—	—	—	—
7DHD	—	0.07 ± 0.03	—	—	—	—
Total sterols	4.49 ± 0.29	4.15 ± 1.54	5.08 ± 3.44	2.68 ± 0.51	3.57 ± 1.13	2.59 ± 0.93
% 7DHC + 8DHC	—	64.06 ± 9.04	—	71.48 ± 11.04	—	53.09 ± 15.04

7DHC, Δ7-dehydrocholesterol; 8DHC, Δ8-dehydrocholesterol; 7DHD, Δ7-dehydrodesmosterol.
—, not measurable.

^{*} *P* < 0.05 compared to *Dhcr7^{+/-}* pups.