Abstract  Elevated plasma LDL cholesterol is the dominant risk factor for the development of atherosclerosis and cardiovascular disease. Deficiency in the LDL receptor (LDLR) is a major cause of familial hypercholesterolemia in humans, and the LDLR knockout mouse is a major animal model of atherosclerosis. Here we report the generation and characterization of an ldlr mutant zebrafish as a new animal model to study hypercholesterolemia and vascular lipid accumulation, an early event in the development of human atherosclerosis. The ldlr mutant zebrafish were characterized by activated SREBP-2 pathway and developed moderate hypercholesterolemia when fed a normal diet. However, a short-term, 5-day feeding of ldlr mutant larvae with a high-cholesterol diet (HCD) resulted in exacerbated hypercholesterolemia and accumulation of vascular lipid deposits. Lomitapide, an inhibitor of apoB lipoprotein secretion, but not the antioxidant probucol, significantly reduced accumulation of vascular lipid deposits in HCD-fed ldlr mutant larvae. Furthermore, ldlr mutants were defective in hepatic clearance of lipopolysaccharides, resulting in reduced survival. Taken together, our data suggest that the ldlr knockout zebrafish is a versatile model for studying the function of the LDL receptor, hypercholesterolemia, and related vascular pathology in the context of early atherosclerosis.

Supplementary key words  low density lipoprotein receptor • atherosclerosis • lipid deposit

The relationship between LDL cholesterol (LDL-C) and risk of cardiovascular disease (CVD) has been well established through numerous epidemiological observational and genetics studies and interventional clinical trials (1). Liver expression of the LDL receptor (LDLR) is a major factor in regulation of plasma levels of LDL-C (2, 3). Human patients with loss-of-function LDLR gene variants develop familial hypercholesterolemia (FH) (1, 4–6), and homozygous FH patients develop advanced atherosclerotic lesions at an early age (4, 5, 7). Expression of LDLR as well as the expression of proteins involved in biosynthesis of cholesterol, such as HMG-CoA reductase (HMGCR), are regulated by the transcription factor SREBP-2, which in turn is regulated by intracellular cholesterol levels (8).

Therapies targeting LDLR expression, such as statins and recently, proprotein convertase subtilisin-kexin type 9 (PCSK9) antibodies, have been successful in lowering LDL-C and reducing incidence of CVD. However, LDLR targeting has its limitations, and there remains a large need for developing drugs targeting non-LDLR pathways. Examples of such non-LDLR pathway therapies include lomitapide, an inhibitor of microsomal triglyceride transfer protein (MTP), and mipomersen, an antisense oligonucleotide targeting apoB mRNA. These two drugs inhibit assembly and secretion of hepatic and intestinal lipoproteins (9). In addition, an antibody targeting angiopoietin-like 3 resulted in a significant reduction of LDL-C in homozygous FH patients (10).

Animal models in which LDLR has been incapacitated may serve to identify new, LDLR-independent targets to treat hypercholesterolemia. Ldlr<−/−> mice (11, 12) have been instrumental in understanding mechanisms of atherosclerosis,
but their use for drug screening is limited because expensive, time-consuming, and labor-intensive procedures are involved in the analysis of atherosclerosis. In contrast, the use of zebrafish is cost-effective because of large progeny numbers and low maintenance costs. Transparent zebrafish larvae are suitable for live imaging, and a quick staining protocol for lipids and easy genetic manipulation allow for effective screening. Recently, new zebrafish models emerged to investigate aspects of lipoprotein metabolism, lipid abnormalities, and atherosclerosis (13–15). Importantly, genes involved in lipid and lipoprotein metabolism, including APOB, APOA family, APOC2, LPL, CETP, and LDLR, are conserved between humans and zebrafish (16–18).

Particularly, LDLr, which is responsible for LDL uptake in liver, has been reported to be functionally conserved in zebrafish. Transient knockdown of ldlr with antisense morpholino oligonucleotides leads to higher LDL-C levels and vascular lipid deposits after feeding a high-cholesterol diet (HCD) (19). To further improve robustness and simplify the experimental procedure, we generated a genetic zebrafish model in which an ldlr mutation achieved by using the CRISPR/Cas9 approach resulted in hypercholesterolemia and robust vascular lipid accumulation following a very short, 5-day HCD feeding. Our data provide evidence that the ldlr mutant zebrafish is a versatile model to study hypercholesterolemia and related vascular pathology.

MATERIALS AND METHODS

Zebrafish maintenance and feeding

Adult zebrafish of the AB strain were maintained at 28°C on a 14 h light/10 h dark cycle and fed brine shrimp twice a day. Zebrafish larvae were fed Golden Pearls (100- to 200-μm size from Brine Shrimp Direct) twice a day, starting from 4.5 days postfertilization (dpf). For HCD feeding, 4% weight per weight (w/w) cholesterol (Sigma, 362794) were added to Golden Pearls, as reported (14, 20). For drug treatment experiments, lomitapide (Cayman, Catalog no. 15043), together with cholesterol, were dissolved in diethyl ether (Sigma, E1010) and thoroughly mixed with Golden Pearls. After diethyl ether evaporation, content of lomitapide was 0.14% w/w, and probucol 0.05% w/w. For visualization of vascular pathology, we generated a genetic zebrafish model in which an ldlr mutation achieved by using the CRISPR/Cas9 approach resulted in hypercholesterolemia and robust vascular lipid accumulation following a very short, 5-day HCD feeding. Our data provide evidence that the ldlr mutant zebrafish is a versatile model to study hypercholesterolemia and related vascular pathology.

CRISPR/Cas9 mediated ldlr knockout in zebrafish

pT3TS-zCas9 and T7-gRNA plasmids were from the Chen lab (21) through Addgene. Following the published protocol, we synthesized nls-zCas9-nls mRNA with a mMESSAGE mMACHINE T3 kit (ThermoFisher, AM1348) and recovered with lithium chloride precipitation. ldlr gRNA was generated using a MEGAshortscript T7 kit (ThermoFisher, AM1354) and purified with a mirVana miRNA isolation kit (ThermoFisher, AM1560). The zebrafish ldlr genomic target sequence was 5′-ggtttgcagcttgctgctg-3′ in which the first 20 nt was the gRNA template and the last 3 nt was protospacer adjacent motif required for CRISPR/Cas9 function. We injected 30 pg ldlr gRNA and 150 pg nls-zCas9-nls mRNA into 1- to 2-cell stage embryos. The genomic DNA (gDNA) was extracted from whole embryos or from adult tail tissue by using a KAPA Express Extract Kit (KAPA Biosystems, KR0383). The gDNA fragment containing the target site was amplified using KOD DNA polymerase (EMD Millipore, 71086) and digested with T7 endonuclease (NEB, M0302). Primers used for PCR amplification of ldlr gDNA fragment were 5′-tcttgagctggacgcttgta-3′ and 5′-tagtgtagcacaattcagac-3′. Primers used for PCR amplification of ldlr cDNA flanking exon 2 to exon 7 were 5′-agttctggggatctaa-3′ and 5′-agttcctggacagctc-3′.

Triglyceride and total cholesterol measurements and lipoprotein analysis

Blood was collected from adult zebrafish, 4 h after feeding, through tail amputation and diluted 1:200 in PBS. Five dpf larvae were gently homogenized in PBS with pestle. After centrifugation, supernatants were collected and referred to as “homogenate.” Triglyceride (TG) and total cholesterol levels in diluted plasma or larvae homogenate were measured with kits according to manufacturer’s protocol (Biovision, Triglyceride Quantification Kit, K622-100; Cholesterol Quantification Kit, K623-100). Lipoprotein fractions were assessed using native agarose gel electrophoresis (Helena Laboratories, 3045) as we previously described (13).

Fast protein liquid chromatography lipoprotein profile

A total of 50 to 60 μl of pooled plasma from 20 to 30 adult WT, ldlr mutant, and apoe2 mutant zebrafish were loaded onto a Superose 6 PC 3.2/30 column (GE Healthcare Life Science, 17-0673-01), and total cholesterol and TG levels were determined in each fraction (250 μl), collected at a flow speed of 0.5 ml/min.

Quantitative RT-PCR

Lipoprotein analysis

RNA isolation was performed using an RNaseasy kit (Qiagen, 74104), and cDNA was reverse transcribed using an EcoRy Premix (Takara-Clontech, 639543). Quantitative PCR (Kapa SYBR FAST qPCR kit, KK4602) was performed using a Rotor Gene Q qPCR machine (Qiagen). Primers used in RT-quantitative PCR were 5′-gagtttcctgcttgatgg-3′ and 5′-ggttgtcagcttgtagtgta-3′ for zebrafish srebp2, 5′-ctcgccttgaacttgaacttg-3′ and 5′-tcgcttacctgcctctctct-3′ for zebrafish apoc2, 5′-cagccaagtgaa-3′ and 5′-agacgacaacaacacaacacaac-3′ for zebrafish srebp1; 5′-aaggagagttcaagcagga-3′ and 5′-gtagggagagcctgtagtgta-3′ for zebrafish srebpl2, 5′-ctgcgcttacctgcctactg-3′ and 5′-tcgcttacctgcctctct-3′ for zebrafish ldlr, 5′-catactggcttacttctcttaagta-3′ and 5′-ctgcgcttacctgcctacctc-3′ for zebrafish fasn, and 5′-ggaaccacaccttaactc-3′ and 5′-caggccgcttactc-3′ for zebrafish pkb9.

Oil Red O and BODIPY staining

For Oil Red O (ORO) staining, embryos or larvae were fixed in 4% paraformaldehyde for 2 h, washed three times in PBS, incubated in 0.3% ORO solution for 2 h, and then washed with PBS before imaging. For BODIPY staining, live larvae were immersed in E3 water (5.0 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl2, 0.33 mM MgSO4) containing 0.1 μg/ml BODIPY 350/515 (Invitrogen, D-3921) for 1 h in the dark and then rinsed with E3 water before imaging.

LPS treatment

Native Escherichia coli O111:B4 lipopolysaccharide (LPS) (Invitrogen, L4130), Pseudomonas aeruginosa LPS (Invitrogen, L9143),
and a fluorescent conjugate of LPS (Alexa Fluor 568-LPS/ Alexa-LPS, Invitrogen, L23352) were dissolved in PBS to a stock concentration of 20 mg/ml. For intravenous LPS injection, 4.5 dpf embryos were laterally aligned in 0.5% low melting temperature agarose, and 5 ml of Alexa-LPS (1 mg/ml) were injected into the circulation through the cardinal vein using a Femtojet microinjector (Eppendorf). For the LPS survival challenge, 5 dpf larvae were immersed in water with different concentrations of E. coli LPS and P. aeruginosa LPS (100–1,000 μg/ml). Larvae survival, assessed by detectable heartbeat, was determined every hour after the immersion.

Imaging of live embryos or larvae

For in vivo microscopy, anesthetized embryos or larvae were mounted in 0.5% low melting temperature agarose (Sigma) containing tricaine (0.02%, Sigma) in 50 mm glass-bottom dishes (MatTek). Images were captured with a Leica CTR5000 or a BZ9000 Keyence fluorescent microscope. To quantify vascular lipid deposits, we captured a z-stack of 15 images in the trunk/tail region with a ×20 objective (BZ9000 Keyence). After overlaying images in the z-stack, using the BZ-X analyzer software (Keyence), vascular lipid deposits were manually counted in five consecutive vascular segments posterior to the anus.

**RESULTS**

**Generation of an ldlr mutant with the CRISPR/Cas9 system**

To create a loss-of-function ldlr mutant in zebrafish, we chose to use the CRISPR/Cas9 system (21). In zebrafish, there are two ldlr orthologs: ldlra (NCBI gene ID: 387529) and ldlrb (NCBI gene ID: 393460), with ldlra showing the higher degree of conservation with the human LDLR gene. The protein sequences between zebrafish Ldlra and human LDLR are conserved with an identity of 51.7% (supplemental Fig. S1), and the zebrafish Ldlra is predicted to have the conserved ligand-binding domain, epidermal growth factor precursor homology domain, and transmembrane domain. Hereinafter in this article, we designate ldlra as the ldlr gene in zebrafish. To disrupt Ldlr protein function with CRISPR/Cas9, we targeted the 5-prime of the ldlr coding region. Five ldlr genomic targets were chosen, and their corresponding gRNAs were synthesized. With T7 endonuclease digestion assay, we found one target, which is localized in exon 5, that was efficiently edited after Cas9/gRNA injection. The F0

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**Fig. 1.** Generation of an ldlr mutant with the CRISPR/Cas9 system. A: The genomic target site of CRISPR is located in exon 5. The heterozygotes from F1 outcross were identified by T7 endonuclease digestion. Black arrows point to heterozygotes. B: Sequences for a selected ldlr mutant, showing a 10nt deletion, which results in frame shift and prestop codon. C: Zebrafish Ldlr protein contains conserved ligand-binding domain, epidermal growth factor precursor homology domain, and transmembrane domain. The C-terminal domains required for Ldlr function are lost in the predicted truncated Ldlr mutant protein. D: cDNAs amplified from adult WT and ldlr mutant liver with primers flanking ldlr exon 3 and exon 7 have a single band of the similar size, indicating no alternative ldlr splicing transcripts in ldlr mutants. EGFP, epidermal growth factor precursor homology domain; LBD, ligand-binding domain; TM, transmembrane domain.
founder fish were raised and outcrossed with WT to obtain F1 generation. F1 were further outcrossed with WT to obtain F2 heterozygotes carrying the same mutation (Fig. 1A). In F1 generation, we screened the \textit{ldlr} mutants and found one mutant line in which a fragment of 10nt was deleted, leading to a prestop codon (Fig. 1B). The truncated protein caused by the 10nt deletion and prestop codon in the \textit{ldlr} mutant lost the whole C-terminal region, which is required for normal LDLR protein function (Fig. 1C). It is reported that frameshift indels may lead to in-frame exon skipping and alternative splicing (22). To check whether this 10nt deletion in exon 5 affected the splicing of the \textit{ldlr} transcript, we designed primers for exon 3 and exon 7 to include exon 5 and the two neighboring exons. Semiquantitative PCR results from adult liver indicated decreased \textit{ldlr} expression but no alternative splicing in the \textit{ldlr} mutant (Fig. 1D), validating the predicted prestop codon. Therefore, this mutant line with the 10nt deletion was defined as our loss-of-function \textit{ldlr} mutant and used in the following studies.

**Hypercholesterolemia and activation of hepatic SREBP-2 pathway in adult \textit{ldlr} mutants**

It is well established that mutations in the LDLR C-terminal region that disrupt LDLR anchoring or processing cause FH in human patients (4). To test whether truncated zebrafish Ldlr protein affects cholesterol homeostasis, we measured plasma lipid levels of the progenies from \textit{ldlr} heterozygotes in-cross. Consistent with human and mouse studies, adult zebrafish homozygous \textit{ldlr} mutants had significantly higher total cholesterol levels than did WT or \textit{ldlr} heterozygote siblings when fed normal diet (Fig. 2A). Plasma triglyceride levels tended to be higher in homozygous \textit{ldlr} mutants but were not significantly different from those in WT or heterozygotes (Fig. 2B). In agreement, neutral lipid staining of plasma separated on native gel suggested higher LDL, but lower VLDL in \textit{ldlr} mutants, when compared with WT (Fig. 2C). Fast protein liquid chromatography data indicated that \textit{ldlr} mutants had a much higher cholesterol levels in the IDL/LDL fractions. In contrast, \textit{apoc2} mutants, reported in our earlier work (13) and used here as a control, showed higher cholesterol levels in the VLDL fraction (Fig. 2D). The TG levels were mildly increased in the LDL fraction in \textit{ldlr} mutants but dramatically increased in the VLDL fraction in \textit{apoc2} mutants, as was expected (Fig. 2E).

The LDLR is required for extracellular cholesterol uptake and regulation of the SREBP-2 pathway in the liver, the major organ responsible for plasma cholesterol regulation (3, 8). We next isolated liver from adult males and

![Fig. 2. Hypercholesterolemia and activation of hepatic SREBP-2 pathway in adult \textit{ldlr} mutants fed normal diet. A, B: Plasma total cholesterol and triglycerides were measured in adult WT, \textit{ldlr} mutant heterozygotes, and homozygotes, which were siblings in the same clutch, genotyped by T7 endonuclease digestion and sequencing (n = 6 for WT, n = 4 for \textit{ldlr} hetero, and n = 3 for \textit{ldlr} homo). C: Native electrophoresis and neutral lipid staining of plasma from adult WT and \textit{ldlr} mutants. D, E: Fast protein liquid chromatography lipoprotein profile of pooled plasma from WT (30 animals), \textit{ldlr} mutant (25 animals), and \textit{apoc2} mutant (20 animals) zebrafish. F: Gene expression of \textit{ldlr}, \textit{srebap2}, and \textit{hmgcr} in the liver of adult males (n = 4 in each group). FPLC, fast protein liquid chromatography. Mean ± SEM; non-significant, \(P > 0.05\); **\(P < 0.01\); ***\(P < 0.001\) (Student’s \(t\)-test).]
extracted total RNA for qPCR. The ldlr mRNA levels were reduced by 75% in ldlr mutants, likely due to nonsense-mediated mRNA decay (23). Interestingly, although srebp2 expression itself was not significantly changed, expression of hmgcr, the gene encoding HMG-CoA reductase and a major target gene of SREBP-2, was increased by as much as 13-fold (Fig. 2F).

Hyperlipidemia and activation of SREBP-2 pathway in ldlr mutant larvae

We next tested whether the ldlr mutant larvae had hyperlipidemia. Compared with WT, 5 dpf ldlr mutant larvae showed stronger vascular ORO and BODIPY staining (Fig. 3A, B), indicating elevated neutral lipid levels in the circulation. In agreement, total cholesterol and triglycerides in larvae homogenates were significantly higher in ldlr mutants than in WT (Fig. 3C, D). To test SREBP-2 activation, we extracted total RNA for qPCR from 4 dpf WT and ldlr mutant larvae. There were no significant changes in srebp1 or srebp2 mRNA expression. However, there was a 4-fold increase in hmgcr expression and a 24-fold increase in fatty acid synthase (fasn) expression in ldlr mutants. Interestingly, expression of pcsk9, another SREBP-2 target, was not changed in ldlr mutants (Fig. 3E).

Increased vascular lipid accumulation in ldlr mutant larvae following short-term high-cholesterol feeding

We and others used zebrafish larvae to monitor vascular lipid accumulation in vivo (13, 14, 19, 20). In our previous studies, WT embryos were fed HCD for 2 weeks before

Fig. 3. Hyperlipidemia and activated SREBP-2 pathway in ldlr mutant larvae. A: ORO and BODIPY staining in 5 dpf WT and ldlr mutant larvae. Scale bars = 100 μm. B: Quantification of BODIPY fluorescence intensity in 5 dpf WT and ldlr mutant larvae (n = 9 for WT and n = 11 for ldlr mutants). C, D: Total cholesterol and triglyceride levels in homogenates from 5 dpf WT and ldlr mutants (n = 4 in each group). E: Gene expression of srebp1, srebp2, hmgcr, pcsk9, fasn, and ldlr in the 4 dpf whole embryo (n = 4 in each group). Mut, mutant. Mean ± SEM; ns, P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001 (Student’s t-test).
imaging vascular lipid deposits (14). Considering the hypercholesterolemia in nonfed ldlr mutant larvae, we tested whether an even shorter-term HCD feeding would induce vascular lipid accumulation. WT and ldlr mutants were fed control diet or HCD starting at 4.5 dpf. After a 5-day feeding, 9 dpf live larvae were immobilized in low-melting temperature agarose and imaged, as described (14, 20). When fed control diet, ldlr mutant larvae showed a moderate but significant increase in vascular lipid deposits in comparison with WT. When fed an HCD, ldlr mutant larvae displayed a dramatic increase in lipid deposits. In contrast, WT larvae did not accumulate vascular lipid deposits within this short time frame of HCD challenge (Fig. 4A, B). There were no apparent morphological or body-size changes in either WT or ldlr mutant larvae following HCD feeding (supplemental Fig. S2).

Our results indicate that the ldlr loss-of-function mutation has resulted in an increased susceptibility to a dietary cholesterol challenge, closely resembling the phenotypes observed in Ldlr<sup>−/−</sup> mice (11, 12). To evaluate whether the new animal model in which ldlr mutant zebrafish are subjected to short-term (5 days) HCD feeding can be useful for drug screening, we tested effects of probucol, an antioxidant, and lomitapide, an MTP inhibitor, on vascular lipid accumulation. Both probucol and lomitapide have been shown to exert antioxidant and MTP inhibitor properties, respectively, in zebrafish (20, 24). As was expected, lomitapide decreased the plasma lipid levels in ldlr mutants, as assessed by ORO staining (Fig. 5A), by blocking their absorption in the intestine. Vascular lipid deposits were not decreased by probucol treatment but were significantly decreased by the treatment with lomitapide (Fig. 5B, C), suggesting that lipid levels, but not lipid oxidation, play a dominant role in early vascular lipid accumulation event in loss-of-function ldlr mutant larvae.

Decreased hepatic clearance of LPS in ldlr mutant larvae

LPS are found in the outer membrane of gram-negative bacteria and elicit strong immune response in animals, in extreme cases resulting in septic shock (25). Previous studies have shown that hepatocytes are responsible for plasma LPS clearance, and this process is dependent on LDLR and its regulator, PCSK9 (26, 27). We tested these hypotheses using our new ldlr mutant zebrafish. At 4.5 dpf, WT and ldlr larvae were injected with red fluorescent Alexa-LPS. After 2 days, fluorescence signals were detected in both liver and vasculature (Fig. 6A). A ratio of Alexa-LPS fluorescence intensity in the liver to that in the vasculature was used as an index for hepatic LPS uptake. The ldlr mutant larvae had a decreased hepatic uptake of Alexa-LPS in comparison with WT (Fig. 6B). To test whether decreased hepatic clearance of LPS affects tolerance of zebrafish larvae to LPS challenge, we tested larvae survival following LPS exposure. Consistent with a previous report (28), we found that P. aeruginosa LPS induced more larval death than did the same doses of E. coli LPS (supplemental Fig. S3). The ldlr mutant larvae exposed to P. aeruginosa LPS had a lower survival than did WT (Fig. 6C).

These results suggest that Ldlr-mediated hepatic clearance of LPS is important for zebrafish survival.

**DISCUSSION**

The goal of this work was to establish a genetic model of hypercholesterolemia in zebrafish. The ldlr mutant zebrafish developed in this study adds to the set of zebrafish models of lipid abnormalities and lipoprotein oxidation, which includes apoC2 mutant zebrafish that develop severe hypertriglyceridemia (13), loss and gain of function liver X receptor mutants (29, 30), and hsp70:IK17-EGFP zebrafish that serve as a reporter for oxidation-specific epitopes (20), among others. We have previously been able to achieve hypercholesterolemia in WT zebrafish by feeding larvae an HCD for 2 weeks, which resulted in accumulation of vascular lipid deposits (14). This approach was further advanced by the introduction of a protocol in which an antisense morpholino oligonucleotide-mediated knockdown of ldlr allowed for shorter HCD feeding times to achieve meaningful hypercholesterolemia and vascular lipid accumulation (19). The introduction of our new ldlr mutant reduces the
variability associated with morpholino oligonucleotide injections and feeding protocols and allows for achieving a robust and consistent phenotype.

The mutation we introduced into the zebrafish ldlra gene created a stop codon and resulted in a truncated, non-functional, and rapidly degraded ldlr transcript. The ldlr

Fig. 5. Effects of probucol and lomitapide on hyperlipidemia and vascular lipid accumulation. A: ORO staining of 9 dpf larvae, following 5-day feeding with HCD, supplemented with probucol and lomitapide. Green arrows point to dorsal aorta, and yellow arrows point to intestine. Scale bar = 500 μm. B, C: Representative images and quantitative data for vascular lipid deposits. Scale bar = 50 μm. Mean ± SEM (n = 8–10 in each group). ns, P > 0.05; **P < 0.01; ***P < 0.001 (Student’s t-test).

Fig. 6. Reduced hepatic uptake of LPS and survival of ldlr mutants upon LPS challenge. A: Representative images of 6.5 dpf WT and ldlr mutant larvae following Alexa-LPS injection (5 nl, 1 mg/ml) at 4.5 dpf. White and green arrows point to fluorescent signals in liver and the tail vasculature, respectively. Scale bars = 200 μm. B: Quantification of the ratio of fluorescent intensity in liver to that in vasculature. Mean ± SEM (n = 6 for WT and n = 5 for ldlr mutant). **P < 0.01 (Student’s t-test). C: Survival curves for 5 dpf WT and ldlr mutant larvae following immersion in water with 500 μg/ml P. aeruginosa LPS (n = 13 for WT and n = 18 for ldlr mutants). P < 0.05 (Log-rank Mantel-Cox test).
vascular lipid deposits. We propose that this new animal feeding results in robust and consistent accumulation of hypercholesterolemia and early atherogenesis in which the LDL particle size and its vulnerability to oxidation.rupted hepatic uptake of LDL in

We also suggest that the advantage of using zebrafish is in the close similarity between zebrafish and human lipoprotein metabolism, as well as the differences that increase zebrafish propensity to developing hypercholesterolemia. Unlike mice, zebrafish express cholesteryl ester transfer protein, which helps retain cholesterol esters in the circulation. In addition, though mouse ApoB proteins are mostly ApoB48, which causes a rapid hepatic cycling of VLDL and low-atherogenic LDL levels (31), zebrafish do not express the apoB mRNA editing enzyme APOBEC1 (apolipoprotein B mRNA editing enzyme 1), and the majority of zebrafish ApoB protein is likely ApoB100, as our earlier studies suggest (32). ApoB100-containing LDL and VLDL remnants have a longer half-life time in plasma, adding to their atherogenicity. Indeed, the lipoprotein profile of normal diet-fed ldlr mutants shows a substantial LDL/LDL fraction, surpassing that seen in chow-fed Ldlr+/− mice. This may explain, in part, why ldlr zebrafish larvae are so sensitive to HCD feeding and accumulate vascular lipid deposits within a short-time frame.

Interestingly, we found that HMG-CoA reductase was consistently and dramatically upregulated in ldlr mutant. However, other srebp2 genes, including pck9, were not significantly changed. We propose that different target genes are regulated by SREBP-2 in a different manner, and it will be intriguing to explore the underlying mechanisms, which might provide new insights and suggest new approaches for differential targeting of genes regulating cholesterol homeostasis. Another interesting observation was the lack of the effect of probucol treatment on vascular lipid accumulation in 5-day HCD-fed ldlr mutants. This was in contrast to our previous results with 15-day HCD-fed WT zebrafish (20). This discrepancy may be explained, in part, by the different time scale of these experiments and disrupted hepatic uptake of LDL in ldlr mutants, which affects the LDL particle size and its vulnerability to oxidation.

In summary, this work introduces a new genetic model of hypercholesterolemia and early atherogenesis in which the ldlr zebrafish mutants subjected to a short, 5-day HCD feeding results in robust and consistent accumulation of vascular lipid deposits. We propose that this new animal model can be used for mechanistic studies and for the screening of new therapeutic targets and treatments.

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