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GENETICS (AJ MARIAN, SECTION EDITOR)

Sortilin as a Regulator of Lipoprotein Metabolism

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Abstract Elevated low-density lipoprotein cholesterol (LDL-C) is associated with increased risk of atherosclerotic cardiovascular disease (ASCVD) and myocardial infarction (MI). Much of the insight into LDL metabolism has been gained through the study of Mendelian disorders of lipid metabolism. Genome-wide associations studies (GWAS) are now being used to identify novel genes and loci that contribute to variations in LDL-C levels, and they have identified the *SORT1* gene as an important modulator of LDL-C levels and ASCVD risk. Mechanistic studies in mice and cell culture also suggest that the *SORT1* gene is an important regulator of lipoprotein metabolism; however, these studies disagree on the directionality of the effect of *Sort1* expression on plasma lipids and the mechanism for the lipid changes. Here we review the identification of the *SORT1* locus as a modulator of LDL-C levels and ASCVD risk and the first mechanistic studies that explore the role of Sortilin in lipid metabolism.

Keywords *Sort1* · Genome wide association study · Atherosclerotic cardiovascular disease · Low-density lipoprotein cholesterol · Lipid metabolism

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Introduction

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of morbidity and mortality in the developed world [1]. The development of ASCVD is strongly influenced by environmental factors including smoking, sedentary lifestyle, obesity, and alcohol consumption, and also by genetic factors, which contribute to the medical risk factors associated with ASCVD development including hypertension, diabetes, elevated low-density lipoprotein cholesterol (LDL-C) and reduced high-density lipoprotein cholesterol (HDL-C). LDL-C is intimately involved in each step in the cascade to cardiovascular disease development: macrophage engorgement and foam cell formation, arterial wall injury, inflammation and smooth muscle cell proliferation, and reduced LDL-C concentrations are associated with protection from ASCVD [2]. In spite of the aggressive use of LDL-lowering medications such as statins, many patients cannot achieve the therapeutic LDL-C levels recommended by clinical guidelines and are thus at risk of developing ASCVD [3]. There is therefore a need for novel therapies to reduce plasma LDL-C and prevent the development of ASCVD and its sequela of myocardial infarction (MI).

Initially, much of the insights into lipid metabolism came from the study of rare Mendelian disorders of high and low LDL-C (Table 1) [4]; however, much of the variation in plasma lipid levels within a population cannot be explained by the rare mutations associated with Mendelian lipid disorders. Genome-wide association studies (GWAS), which involve genotyping hundreds of thousands of single nucleotide polymorphisms (SNPs) in thousands of individuals phenotyped for lipid traits, have evolved as an unbiased tool to identify common variants that contribute in a causal manner to lipid variations in the population [5, 6, 7•, 8•]. Importantly, GWAS aims to identify many genes and loci of generally

Table 1 Summary of Mendelian disorders of elevated and reduced LDL-C

Disease	Phenotype	Causal Gene	Mutation
Familial hypercholesterolemia	Tendon xanthomas, elevated plasma LDL-C, premature ASCVD	LDLR	Loss of function
		PCSK9	Gain of function
		APOB	Loss of function
Autosomal recessive hypercholesterolemia	Elevated plasma LDL-C and premature ASCVD	LDLRAP	Loss of function
Hypobetalipoproteinemia	Reduced plasma LDL-C	APOB	Loss of function
		PCSK9	Loss of function
Abetalipoproteinemia	Absent apoB-containing lipoproteins, acanthocytosis, ataxia	MTTP	Loss of function
Sitosterolemia	Elevated plant sterols, elevated LDL-C, premature ASCVD	ABCG5/8	Loss of function

apoB apolipoprotein B; *ASCVD* atherosclerotic cardiovascular disease; *LDL-C* low-density lipoprotein cholesterol

small effect size that contribute to population variations in a trait, instead of single gene defects that have dramatic effects on phenotype, like those seen in Mendelian disorders.

GWAS for lipid traits have already identified common variants in genes known to cause Mendelian lipid disorders, including *LDLR*, *PCSK9*, *APOB*, *LDLRAP*, and *IBCG5/8* for LDL-C. GWAS have also identified genes that are drug targets for LDL-lowering therapies, including *NPC1L1*, the molecular target of ezetimibe, and *HMGCR*, the molecular target of statin drugs [8•]. GWAS have also identified a number of novel genes and loci that contribute to population variability in LDL-C. The locus in the human genome with the strongest association to LDL-C is a novel 1p13 locus, which harbors the genes *SORT1*, *PSRC1*, and *CELSR2*. Importantly, the 1p13 locus is associated not only with the intermediate phenotype of LDL-C, but also with the hard clinical endpoint of cardiovascular disease. This review focuses on the initial identification of the 1p13 locus as a genome-wide significant and important determinant of LDL-C levels and cardiovascular disease risk in the population, early mechanistic studies to determine how the 1p13 locus affects LDL-C and cardiovascular disease risk, and the current controversy surrounding the disparate mechanistic studies that have emerged.

Initial Identification of the *SORT1* Locus

Before its association with LDL-C levels in humans, the 1p13 locus was identified in 2007 in the Welcome Trust Case Consortium (WTCCC) as an important risk factor for coronary artery disease Table 2 [9]. The association between the 1p13 locus and cardiovascular disease was replicated in other larger studies, including the Global Lipids Genetics Consortium, which included more than 100,000 individuals of European decent [8•], and most recently in the CARDIoGRAM study, which included more than 140,000 individuals of European decent [10•] . The association between the 1p13 locus and cardiovascular disease has been replicated in non-European populations, as well [8•, 11, 12]. The 1p13 locus has also been linked to MI, and homozygosity for the minor alleles at 1p13 is associated with a 40% reduction in MI risk [9, 13•].

Shortly after the 1p13 locus was found to be associated with cardiovascular disease, a large-scale GWAS with 9,000 individuals of European decent and a replication study in 18,000 individuals of European decent identified the 1p13 locus as a genome-wide significant determinant of LDL-C levels in humans [5]. Importantly, this study demonstrated that homozygosity for the minor allele is associated with a 16 mg/dL lower systemic LDL-C as compared to homozygotes for the major allele, which is an unusually large effect size for a common variant. These results have been confirmed in other European and non-European populations [14, 15•, 16]. All subfractions of LDL were reduced in individuals of the minor haplotype; however, the most dramatic reductions occurred in the small, dense LDL fractions, which are derived from the lipolysis of triglyceride-rich VLDL1 and thought to be the most atherogenic [17•].

The 1p13 locus has been replicated in larger GWAS for lipid traits in European populations, including a study with 40,000 individuals, and the largest GWAS for lipid traits, the Global Lipids Genetics Consortium (GLGC), which included over 100,000 individuals of European descent. The GLGC study named the 1p13 locus as the most strongly associated with LDL-C in European populations, with a P value of 1×10^{-170} (95% CI, 5.24–6.06 per allele copy) [8•]. Importantly, the 1p13 locus has been replicated as an important determinant of LDL-C in non-European populations as well [8•, 16, 18, 19].

Dissecting the Locus and the Association Signal

The challenge of the 1p13 locus is that the SNPs most strongly associated with LDL-C and ASCVD risk lie in a non-coding region between two genes, *PSRC1* and *CELSR2*, and lie extremely close to the *SORT1* gene, making it difficult to name the gene responsible for the

association. Expression quantitative trait loci (eQTL) data suggest that homozygosity for the minor allele is associated with elevated expression of *SORT1*, *PSRC1*, and *CELSR2* in human liver, with *SORT1* and *PSRC1* expression most strongly influenced by genotype with 10- to 12-fold elevations in expression with homozygosity for the minor allele. Interestingly, the expression changes seen with homozygosity for the minor allele were not present in subcutaneous or omental adipose, suggesting a liver-specific effect of the 1p13 locus on LDL-C levels and ASCVD risk [17•].

Further complicating the problem is that none of these genes have an obvious link to LDL metabolism: *PSRC1* encodes the protein proline-serine rich coiled-coil domain-1, which is a downstream target of p53 and is believed to be involved in cell cycle progression and formation of the mitotic spindle. *CELSR2* encodes the protein cadherin EGF LAG seven-pass G-type receptor 2, which is involved in neuronal cell migration and adhesion. Neither *PSRC1* nor *CELSR2* are well-characterized proteins. This contrasts with *SORT1*, which encodes the protein sortilin, which is fairly well characterized as a cell surface receptor as well as an intracellular trafficking receptor.

Sortilin is a multi-ligand sorting receptor originally identified by receptor-associated protein (RAP) affinity chromatography [20]. Sortilin is one of five members of the mammalian VPS10 domain family and harbors an N-terminal propeptide with a furin cleavage site, an extracellular VPS10 domain for ligand binding, a single transmembrane domain, and a C-terminal cytoplasmic tail with strong homology to the cation-independent mannose-6-phosphate receptor (CI-M6PR), bearing two endolysosomal sorting motifs: a dileucine-based sorting motif and a tyrosine-based sorting motif [20].

Sortilin localizes primarily to the Golgi apparatus and has been shown to bind a variety of ligands there and traffic them to the lysosome (Fig. 1). Sortilin also localizes to the plasma membrane where it can act as a signalling receptor for pro-neurotrophins [21], function as an uptake receptor [22•], or be cleaved by the metallopeptidase TNF alpha-converting enzyme (TACE) in a mitogen-activated protein kinase (MAPK)/protein kinase C (PKC)-dependent manner to generate a soluble fragment comprising the sortilin luminal domain [23]. It has recently been reported that sortilin is cleaved intracellularly and at the cell surface by the protease ADAM10 to generate the soluble luminal domain [24].

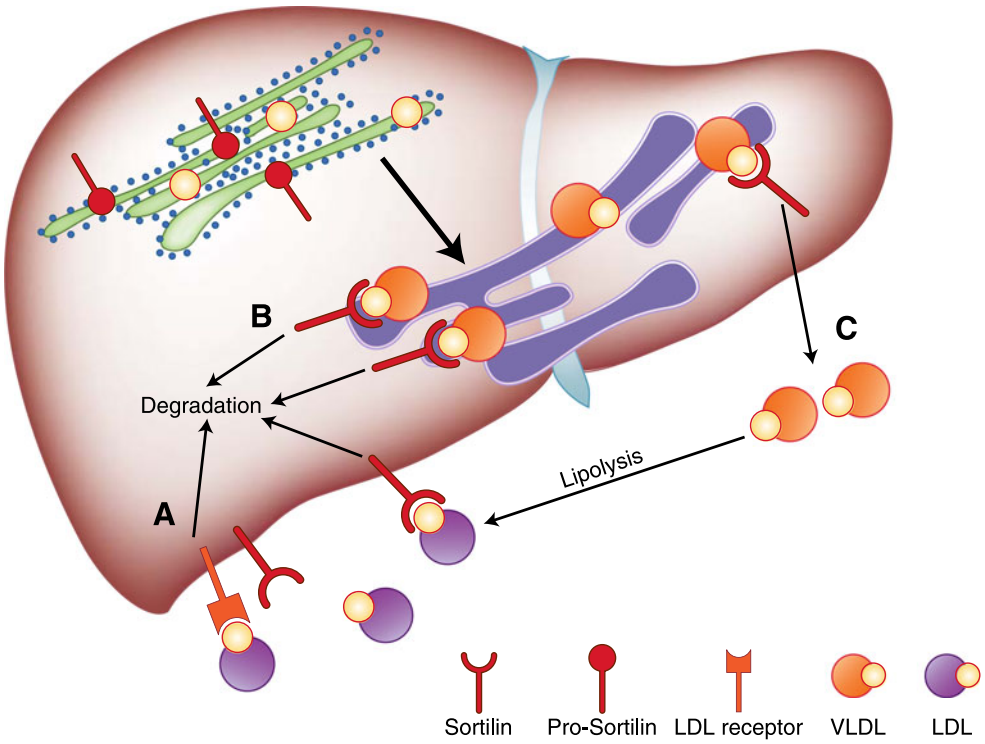

Fig. 1 Sortilin, the protein product of the *SORT1* gene, is synthesized as a proprotein and is cleaved by furin in the trans-Golgi network to produce the mature sortilin protein. Very low-density lipoprotein (VLDL) synthesis begins in the rough endoplasmic reticulum (RER, *pink*) with synthesis and lipidation of its primary protein component, apolipoprotein B (apoB, *yellow circle*). apoB is further lipidated and modified in the Golgi apparatus (*blue*) to form a mature VLDL particle. VLDL can either undergo pre-secretory degradation or can be secreted. VLDL is lipolyzed in the periphery to generate LDL, which is taken up by liver primarily by the LDL receptor. **a** Work by Linsel-Nitschke et al. [15•] suggests that *Sort1* overexpression reduces LDL-C by enhancing uptake of low-density lipoprotein (LDL) particles in vitro. **b** Work by Musunuru et al. [17•] suggests that *Sort1* expression reduces LDL-C by reducing production/secretion of the LDL precursor VLDL cholesterol. **c** Work by Kjolby et al. [28•] suggests that *Sort1* overexpression increases LDL cholesterol by facilitating secretion of the LDL precursor VLDL

Sortilin binds to a number of ligands involved in lipid metabolism including lipoprotein lipase [25], apolipoprotein E [26], and apolipoprotein AV [27], which are involved in triglyceride metabolism. It is unclear, however, how these interactions might explain the strong association of the *SORT1* locus with LDL-C.

Identification of *SORT1* as the Causal Gene and Early Mechanistic Studies

One year following identification of the 1p13 locus as a genetic determinant of LDL-C levels and ASCVD risk, the first mechanistic study was published. Linsel-Nitschke et al. [15•] replicated the association of the 1p13 locus with LDL-C and CAD risk and further demonstrated that homozygosity for the minor allele was associated with elevated expression of *Sort1* in peripheral blood with no change in *PSRC1* or *CELSR2* expression. The directionality was consistent with the eQTL data that associated minor allele homozygosity with elevated *SORT1* expression in liver and supported a role for *SORT1* as the causal gene at the 1p13 locus responsible for the LDL association.

To determine whether *SORT1* overexpression affects LDL-C levels by enhancing LDL catabolism, Linsel-Nitschke et al. [15•] transfected HEK293 cells with a cDNA encoding human *SORT1* and assayed for LDL uptake. They incubated cells with ^{125}I-LDL for 4 h in the presence or absence of competitive inhibitors followed by counting intracellular radioactivity. Consistent with the lower plasma LDL-C seen in humans with elevated *Sort1* expression, *SORT1* overexpression in vitro caused a small but statistically significant increase in LDL uptake [15•]. This study supported *SORT1* as the causal gene at the 1p13 locus and suggested a mechanism by which elevated *SORT1* expression in human liver could reduce LDL-C and ASCVD risk, namely by increasing LDL uptake in liver. However, this work fell short of identifying the mechanism by which *SORT1* expression could enhance LDL uptake.

Table 2 Genome-wide association of the 1p13 *SORT1* locus with LDL-C and cardiovascular disease

Trait	Year	Ancestry	Reference
LDL-C	2008	European	[5, 6]
LDL-C	2009	Japanese	[17•]
LDL-C	2009	Non-Hispanic blacks	[16]
LDL-C	2009	Non-Hispanic whites	[16]
LDL-C	2009	Mexican Americans	[16]
LDL-C	2009	European	[7•]
LDL-C	2010	African	[18•]
LDL-C	2010	European	[8•]
LDL-C	2010	East Asian	[8•]
LDL-C	2010	South Asian	[8•]
LDL-C	2010	African American	[8•]
LDL-C	2011	Chinese Han	[12]
Coronary artery disease	2007	European	[9]
Coronary artery disease	2010	European	[8•]
Myocardial infarction	2009	European	[13•]
Myocardial Infarction	2011	Hispanic	[11]
Cardiovascular disease	2011	Chinese Han	[12]
Cardiovascular disease	2011	European	[10•]

LDL-C low-density lipoprotein cholesterol

Identification of the Causal SNP and the Mechanism of *Sort1* Overexpression

Homozygosity for the minor allele at the 1p13 locus is associated with a 10- to 12-fold increase in *SORT1* and *PSRC1* expression with a significant eQTL in human liver; however, when the GWAS was published, the mechanism for the profound effect on gene expression was unknown. A study published in 2010 by Musunuru et al. [17•] identified the genetic mechanism of the expression changes. Musunuru et al. [17•] looked at expression of all nearby genes in the 1p13 locus (*SARS*, *CELSR2*, *PSRC1*, *MYBPHL*, *SORT1*, *PSMA5*, and *SYPL2*) in liver, subcutaneous adipose and omental adipose in individuals genotyped for the associated haplotype block at the 1p13 locus and found that homozygosity for the minor allele is associated with a 10- to 12-fold increase in expression of *SORT1* and *PSRC1* in human liver with heterozygotes for the minor allele having an intermediate phenotype. *CELSR2* expression also increased with homozygosity for the minor allele; however, the magnitude of the expression change was far less robust. Importantly, homozygosity for the minor alleles at 1p13 did not lead to an expression change in *SORT1*, *CELSR2*, or *PSRC1* in subcutaneous or omental fat, suggesting a liver specificity to the expression changes seen with the protective minor alleles [17•].

Through fine mapping studies, Musunuru et al. [17•] identified a 6.1-kB segment of genomic DNA that contained the causal variant that explains the 1p13 LDL-C association. Musunuru et al. [17•] identified bacterial artificial chromosomes (BACs) containing either the major or minor haplotype and cloned them into a firefly luciferase expression vector, transfected the construct into a hepatic cell line, and showed that the BAC harboring the minor allele haplotype was able to drive higher luciferase expression than the major allele haplotype, suggesting that the 6.1-kB region contained the genetic regulatory element responsible for increased expression at the 1p13 locus conferred by the minor allele. Through a series of deletion and mutagenesis constructs, Musunuru et al. [17•] were able to identify the causal SNP that alone conferred the expression changes of

the minor allele. Through a series of dominant negative C/EBP transfection studies, studies in cells lacking CCAAT-enhancer-binding protein (C/EBP), electrophoretic mobility shift assay (EMSA) studies, and chromatin immunoprecipitation (ChIP) experiments, Musunuru et al. [17•] were able to demonstrate that the minor allele at rs12740374 creates a C/EBP transcription factor binding site that is not present in the major allele. As C/EBP is a liver-enriched transcription factor, this could explain the specificity of the expression changes to liver.

Proof of Principle in Mice: Sortilin Overexpression Reduces Plasma Lipids and VLDL Secretion

To take the story further, Musunuru et al. [17•] used adeno-associated virus (AAV) serotype 8 to drive liver-specific expression of a murine *Sort1* cDNA in mice and looked at the effects on plasma lipids in a variety of "humanized" mouse models of lipoprotein metabolism, including *ApoBEC−/−*; *APOB* transgenic, *ApoBEC−/−*; *LDLR+/−; APOB* transgenic, *ApoBEC−/−; LDLR−/−; APOB* transgenic, and *ApoBEC−/−*; *LDLR−/−* mice. These mouse models have lipid profiles more similar to humans, as human liver does not express *ApoBEC* whereas mouse liver does, and wild-type mice are predominantly HDL animals, whereas humans have much more LDL-C in circulation. In all of these mouse models, sortilin expression consistently reduced plasma cholesterol ranging from 30% to 70%. Musunuru et al. [17•] also used AAV8 to express *Psrc1* in mouse liver in *LDLR−/−* mice, and showed no effect on plasma lipids, again supporting *SORT1* as the causal gene at the 1p13 locus.

To address the mechanism, Musunuru et al. [17•] performed in vivo VLDL secretion studies in *Sort1*-expressing mice and showed a 50% reduction in the VLDL secretion rate with *Sort1* expression. They also performed S35-labelling experiments in primary hepatocytes isolated from control and *Sort1*-expressing mice and showed a 40% reduction in apoB secretion with *Sort1* expression, suggesting that *Sort1* reduces plasma cholesterol by reducing the VLDL secretion rate [17•].

Proof of Principle: Sortilin Knockdown Increases Plasma Lipids and apoB Secretion

As a complementary approach to their overexpression studies, Musunuru et al. [17•] used short-interfering RNAs (siRNAs) directed against *Sort1* prepared as a lipidoid formulation to knock down *Sort1* expression specifically in liver in *ApoBEC−/−; APOB* transgenic, *ApoBEC−/−; LDLR+/−; APOB* transgenic, and *ApoBEC−/−; LDLR−/−* mice. These siRNAs were successful in reducing *Sort1* expression 70% to 90% in liver with no effect on *Sort1* expression in adipose. Consistent with the human data and with their overexpression data, liver-specific *Sort1* knockdown consistently increased plasma cholesterol 20% to 125% [17•].

To determine whether *Sort1* knockdown leads to increased apoB secretion, primary hepatocytes were isolated from control and siRNA-injected *Sort1* knockdown mice and S35-labelling experiments were performed. Consistent with the overexpression studies, *Sort1* knockdown in primary hepatocytes led to a 75% increase in apoB secretion [17•].

Disparate Findings in *Sort1−/−* Mice

Shortly after the Musunuru et al. [17•] study was published, a second study by Kjolby et al. [28•] using a *Sort1−/−* mouse was published. After 6 weeks of western-type diet feeding, Kjolby et al. [28•] showed that *Sort1−/−* mice had 20% lower plasma cholesterol compared to wild-type mice, and *Sort1−/−; LDLR−/−* double knockout mice had 30% lower cholesterol than *LDLR−/−* mice. Kjolby et al. [28•] went on to show that *Sort−/−; LDLR−/−* double knockout mice fed a western-type diet for 8 months had a 60% reduction in atherosclerotic lesion formation. Kjolby et al. [28•] also found that overexpression of a human *Sort1* cDNA in liver by adenovirus in wild-type and *Sort1−/−; LDLR−/−* double-knockout mice fed a western-type diet increased plasma cholesterol [28•]. This contrasts with the human GWAS data and the mechanistic studies done by Linsel-Nitschke et al. [15•] and Musunuru et al. [17•], which all predict that *increased Sort1* expression should *reduce* LDL-C levels deficiency should increase plasma cholesterol and the burden of atherosclerotic disease.

Kjolby et al. [28•] also looked at apoB and triglyceride secretion in primary hepatocytes and live animals and found that *Sort1* deficiency was associated with reduced apoB secretion in primary hepatocytes and reduced apoB and triglyceride secretion in mice. Kjolby et al. [28•] also demonstrated that *Sort1*-deficient mice had increased intracellular apoB content, and this was reduced with adenovirus-mediated *Sort1* overexpression. Kjolby et al. [28•] went on to use immunofluorescence, immunoprecipitation, and surface plasmon resonance to show that sortilin binds apoB with extremely high affinity, with a K_d of 1–2 nM and does not bind to apoB48. They suggested that *Sort1* serves as a facilitator of hepatic apoB100 export, possibly by direct binding to apoB100.

Reconciling the Differences

The disparate findings of Musunuru et al. [17•] and Kjolby et al. [28•] bring into question the true biology of *Sort1* as well

as the therapeutic strategy to harness the LDL-modulating power of the 1p13 GWAS locus. The human GWAS data are in agreement with the studies published by Musunuru et al. [17•] and Linsel-Nitschke et al. [15•] and suggest that therapies that raise hepatic *Sort1* expression will reduce plasma cholesterol and protect against ASCVD either by reducing VLDL secretion, facilitating LDL uptake, or a combination of both. Additionally, work by Ai et al. [29•] has shown that pharmacologic treatments that reduce *Sort1* expression and mouse models that have reductions in *Sort1* expression in liver have increased apoB/VLDL secretion, whereas restoration of *Sort1* expression in mouse liver using AAVs and mouse models with increased *Sort1* expression have reduced VLDL/apoB secretion, consistent with the studies published by Musunuru et al. [17•]. But, is there important biology being shown to us through the findings in the knockout mice?

The human GWAS data suggest that liver-specific increases in *Sort1* expression are protective. The studies by Musunuru et al. [17•] used liver-specific manipulations to determine the effect of *Sort1* overexpression and knockdown on plasma lipids and VLDL secretion, whereas the studies by Kjolby et al. [28•] used a total body *Sort1*−/− mouse. *Sort1* is expressed both in adipose and small intestine, which are critical organs in lipid metabolism, and *Sort1* deficiency in these tissues may be contributing to the lipid and secretory phenotypes observed in the study by Kjolby et al. [28•].

There are also differences in timing, mechanisms of *Sort1* manipulations, diet, and mouse models that could explain the disparate phenotypes observed by the two groups (Table 3). Musunuru et al. [17•] performed acute somatic manipulations of *Sort1* expression using siRNA and AAV, whereas in the Kjolby mouse model *Sort1* has been deficient since conception. Compensatory mechanisms could be in place, which could explain the disparate phenotypes. Furthermore, whereas Kjolby et al. [28•] used a model completely lacking *Sort1*, the siRNAs used by Musunuru et al. [17•] reduced hepatic *Sort1* expression 70% to 90%, meaning there was still residual *Sort1* expression. There may be a parabolic relationship between *Sort1* concentration and VLDL secretion, explaining the disparate findings in the knockout versus the knockdown models.

Also of note is a recent paper using the *Sort1*−/− mouse used by Kjolby that suggests that the mouse is not a complete knockout mouse, but a deletion mutant [22•]. The Kjolby mouse has a disruption at exon 14 [30], which leads to a deletion in the VPS10 domain, but still allows a protein to be made. This protein is misfolded and does not traffic to the cell surface; however, this aberrant protein could potentially explain the disparate results.

Conclusions

The 1p13 locus and the causal gene *SORT1* represent a promising target for LDL lowering and ASCVD risk reduction. Human GWAS data and the functional studies of alleles identified through GWAS performed by Linsel-Nitschke et al. [15•] and Musunuru et al. [17•] suggest that the therapeutic goal should be to raise *Sort1* expression in liver. However, Kjolby et al.'s [28•] findings in the *Sort1*−/− mouse call into question the therapeutic strategy for *Sort1* targeting, and suggest that it is *Sort1* inhibition that will

Table 3 Summary of the in vivo data suggesting that *Sort1* is an important regulator of low-density lipoprotein metabolism

Mouse model	Manipulation	Duration	Diet	Lipid effect	Study	Result predicted by GWAS
ApoBEC−/−; *APOB* TG	AAV Overexpression Murine *Sort1*	2 weeks	Chow	↓ 70%	Musunuru, et al. [17•]	↓
ApoBEC−/−; *LDLR*+/−; *APOB TG*	AAV Overexpression Murine *Sort1*	2 weeks	Chow	↓ 44%	Musunuru, et al. [17•]	↓
ApoBEC−/−; *LDLR*−/−; *APOB TG*	AAV Overexpression Murine *Sort1*	2 weeks	Chow	↓ 60%	Musunuru, et al. [17•]	↓
ApoBEC−/−; *LDLR*−/−	AAV Overexpression Murine *Sort1*	2 weeks	Chow	↓ 26%	Musunuru, et al. [17•]	↓
Wild type	Adenovirus Overexpression Human *Sort1*	2 weeks	Western-type Diet	↑ 42%	Kjolby et al. [28•]	↓
SORT−/−; *LDLR*−/−	Adenovirus Overexpression Human *Sort1*	2 weeks	Western-type Diet	↑ 30%	Kjolby et al. [28•]	↓
ApoBEC−/−; *APOB* TG	siRNA Knockdown	2 weeks	Chow	↑ 46%	Musunuru, et al. [17•]	↑
ApoBEC−/−; *LDLR*+/−; *APOB TG*	siRNA Knockdown	1 week	Chow	↑ 25%	Musunuru, et al. [17•]	↑
ApoBEC−/−; *LDLR*−/−	siRNA Knockdown	1 week	Chow	↑ 24%	Musunuru, et al. [17•]	↑
Sort−/−	Knockout	6 weeks	Western-type Diet	↓ 20%	Kjolby et al. [28•]	↑
Sort−/−;*LDLR*−/−	Knockout	6 weeks	Western-type Diet	↓ 30%	Kjolby et al. [28•]	↑

AAV adeno-associated virus, *GWAS* genome-wide association study; *siRNA* small interfering RNA

reduce plasma cholesterol and ASCVD risk. All research published to date support an unequivocal role for *Sort1* in lipid metabolism and ASCVD and suggest that the power of the 1p13 locus can be harnessed to dramatically affect plasma cholesterol and ASCVD risk in humans. Future studies should aim to clarify the directionality of sortilin's effect on plasma lipids by standardizing the knockout, knockdown, and overexpression studies in terms of diet and mouse model to see if the disparate findings can be reconciled.

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