Brief Review

Sortilin and Its Multiple Roles in Cardiovascular and Metabolic Diseases

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Abstract—Cardiovascular disease is a leading cause of morbidity and mortality in the Western world. Studies of sortilin’s influence on cardiovascular and metabolic diseases goes far beyond the genome-wide association studies that have revealed an association between cardiovascular diseases and the 1p13 locus that encodes sortilin. Emerging evidence suggests a significant role of sortilin in the pathogenesis of vascular and metabolic diseases; this includes type II diabetes mellitus via regulation of insulin resistance, atherosclerosis through arterial wall inflammation and calcification, and dysregulated lipidprotein metabolism. Sortilin is also known for its functional role in neurological disorders. It serves as a key receptor for cytokines, lipids, and enzymes and participates in pathological cargo loading to and trafficking of extracellular vesicles. This article provides a comprehensive review of sortilin’s contributions to cardiovascular and metabolic diseases but focuses particularly on atherosclerosis. We summarize recent clinical findings that suggest that sortilin may be a cardiovascular risk biomarker and also discuss sortilin as a potential drug target.

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Key Words: atherosclerosis ■ cardiovascular disease ■ clinical study ■ metabolic disease ■ morbidity

Genetic Aspects of Sortilin Biology: Relationship Between the Sortilin Locus and Cardiovascular Diseases

Atherosclerosis is a multifactorial disease. A combination of environmental and genetic factors contributes to the development of atherosclerosis. A large number of genome-wide association studies (GWAS) have been conducted in an attempt to identify novel candidate genes or loci involved in the generation of cardiac phenotypes ranging from high circulating levels of low-density lipoprotein (LDL) cholesterol (LDL-C),1-3 myocardial infarction,4,5 and various other aspects of atherosclerosis.6-9 Since 2007, new candidate genes affecting LDL-C have been identified through GWAS, including the 2 loci harboring CILP2-PBX4 and CELSR2-PSRC1-MYBPHL-SORT1.3 Several single-nucleotide polymorphisms are in the region of the gene cluster CELSR2-PSRC1-MYBPHL-SORT1 at 1p13.3. All single-nucleotide polymorphisms are located in the intergenic region between PSRC1 and CELSR2 and downstream of SORT1 and MYBPHL.10 SORT1 encodes sortilin, PSRC1 encodes proline-serine-rich coiled-coil protein 1, MYBPHL encodes myosin-binding protein H like, and CELSR2 encodes cadherin EGF LAG (epidermal growth factor laminin A G-type) seven-pass G-type receptor 2.

The major haplotype block, comprising rs599834, rs646776, rs629301, and rs12740374, associates with elevated LDL-C levels in many cohorts.1,11,12 The association of the genotype with the expression of the gene cluster SORT1-PSRC1-CELSR2 seems to be tissue-specific. Liver-based expression quantitative trait loci revealed that SORT1, CELSR2, and PSRC1 are coregulated in the same direction by the above-mentioned single-nucleotide polymorphisms.1,13,14 Schadt et al15 used 427 human liver samples and demonstrated that the minor (protective) allele of rs599839 associates with increased hepatic SORT1 and CELSR2, and the expression of both genes correlated negatively with LDL-C. In this study, decreased hepatic PSRC1 associated with the minor allele genotype and with decreased LDL-C. Later studies by Kathiresan et al16 and Musunuru et al17 showed that the presence of the minor (protective) allele of rs646776 associated with elevated hepatic expression of SORT1, CELSR2, and PSRC1, and these correlated negatively to LDL-C using 60 and 960 human liver samples, respectively. In other cohorts where the minor allele of rs599839 and rs646776 also associated with decreased LDL-C, the gene cluster SORT1-PSRC1-CELSR2 was analyzed in whole blood. Data from whole-genome expression showed a significant increase in SORT1 expression associated with the minor allele of rs599839, whereas no association was detected between the genotypes and PSRC1 and CELSR2.15 Whole-blood quantitative polymerase chain reaction analysis demonstrated an association of the minor allele with the expression of PSRC1, whereas there

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was no alteration in the expression of SORT1 and CELSR2. Of note, the expression quantitative trait loci effect sizes are much smaller in whole blood than in liver and, therefore, might not be of functional consequence for sortilin biology. Further, analysis of tissue from abdominal aortic aneurysm (n=108) revealed no association of the rs599839 or rs12740374 genotype with sortilin protein expression. The GWAS were not able to pinpoint an exact molecular mechanism of any of the individual genes but showed correlation with LDL-C. Furthermore, the SORT1 locus also associated with the following cardiac phenotypes: coronary artery disease, early-onset myocardial infarction, abdominal aortic aneurism, coronary stenosis, coronary artery calcification, and aortic valve calcification. All these diseases are interconnected and could be linked through LDL-C. Because these studies show association with LDL-C, it makes it difficult to determine whether sortilin has an additional direct effect on these cardiac phenotypes independent of LDL-C. This warrants more research to interrogate the direct mechanistic effects of SORT1 in atherosclerotic plaque development, calcification, and aneurism formation through direct genetic manipulation. Real-time information on the genetic variation of SORT1 and different human tissue gene expression can be found at the database https://gtexportal.org/.

Sortilin in Atherosclerosis

Sortilin is a member of the vacuolar protein sorting 10 protein family of sorting receptors. It is a 110 kDa single-pass type I transmembrane protein initially identified in brain tissue but now shown to be expressed in many cell types, including cardiovascular tissues. Sortilin is synthesized with a propeptide that is necessary for correct folding and inhibition of premature ligand binding. The propeptide is cleaved off in the late part of the trans-Golgi network. Recently, sortilin was recognized as a major player in various processes of atherogenesis. Preclinical in vivo evidence suggests a significant role of sortilin in the pathogenesis of vascular and metabolic disorders, including atherosclerosis, through contributions to arterial wall inflammation and calcification, dysregulated lipoprotein metabolism, and type II diabetes mellitus—all cardiovascular risk factors (Figure).

Inflammation

The first experimental evidence for a link between sortilin and atherosclerosis was provided by Kjolby et al who demonstrated reduced atherosclerotic plaque size caused by global deletion of sortilin in Ldlr (low-density lipoprotein receptor)-deficient mice. This finding was later supported by Patel et al using sortilin-deficient mice in humanized Apobec1(-/-);hAPOB transgenic background.

Both groups performed bone marrow transplantation from sortilin-deficient mice into irradiated Apoe- and Ldlr-deficient mice and showed a reduction in plaque size without changes in total cholesterol and LDL-C. Mechanistic studies revealed a role of sortilin in inflammatory response as well as foam cell formation, whereas macrophage recruitment was not affected. Mortensen et al stimulated bone marrow cells isolated from sortilin-deficient and control mice toward proinflammatory macrophages using lipopolysaccharides and found reduced levels of interleukin (IL)-6 and interferon-γ secreted from sortilin-deficient macrophages and type 1 T-helper cells, whereas other cytokines (eg, tumor necrosis factor-α, IL-12) were similar between groups. Further, IL-6 levels were reduced in plasma of Apoe-deficient mice transplanted with sortilin-deficient cells and fed a Western diet for 9 weeks. Binding assays demonstrated an association of the extracellular domain of sortilin with both cytokines that was abolished by sortilin propeptide, suggesting a binding to the tunnel structure. Patel et al followed an in vivo approach and assessed the lipopolysaccharide-mediated inflammatory response in sortilin-deficient and control mice 2 and 5 hours post-injection and did not find any difference in serum cytokine levels. Interestingly, Sort1/Ldlr-deficient bone marrow–derived macrophages differentiated by macrophage colony-stimulating factor demonstrated reduced foam cell formation after LDL stimulation. These finding were supported by in vivo foam cell formation assays on Sort1-deficient/Apobec1(-/-);hAPOB mice fed a Western diet for 18 weeks. Peritoneal macrophages isolated from Sort1-deficient/Apobec1(-/-);hAPOB mice displayed reduced Oil Red O staining and cellular cholesterol. LDL uptake studies using bone marrow–derived macrophages from sortilin-deficient mice revealed an LDL receptor–independent pathway in sortilin-mediated LDL uptake. Mortensen et al also studied the role of sortilin in LDL uptake using fluorescently-labeled native, aggregated, and oxidized LDL and flow cytometry. There was no difference in LDL uptake between sortilin-deficient bone marrow macrophages and control.

These studies suggest that although sortilin deficiency reduces plaque size, the impact on inflammatory response and foam cell formation varies depending on the mouse model and analytical assays. Whether these different mechanistic findings are attributable to the different mouse genetic backgrounds is unclear and requires further investigation. Further studies on human macrophages are needed to conclude a consequence for translational aspects. It would be imperative to assess the expression of sortilin in human pro- and anti-inflammatory macrophages and the expression levels in human atheroma to draw a conclusion of the importance of sortilin in macrophage biology and provide mechanistic insights into the role of human sortilin in atherosclerosis development.

Dyslipidemia

It is well accepted that plasma lipid levels contribute to the pathogenesis of atherosclerosis. The controversy about sortilin’s role in hepatic lipid metabolism still exists and has been discussed in several reviews and editorials. GWAS implicated sortilin in systemic cholesterol homeostasis. Human and mouse studies implicated hepatic sortilin as a protective protein attenuating circulating cholesterol.
levels. Possible underlying mechanisms include sortilin as a hepatic clearance receptor for LDL and as a sorting receptor for lipoprotein particles that reduced very-low-density lipoprotein secretion. Two independent studies using whole-body sortilin-deficient mouse strains, however, showed reduced cholesterol levels. Proposed sortilin functions associated with increased cholesterol levels include its receptor high affinity for APOB100 to facilitate very-low-density lipoprotein secretion from hepatocytes. In addition, sortilin was also identified as a high-affinity receptor for PCSK9 (proprotein convertase subtilisin/kexin type 9); the latter binds LDL receptor during lysosomal degradation. This is of high clinical significance because pharmacological inhibition of PCSK9 has become important in the treatment of patients with high LDL-C levels. In vivo loss-of-function and gain-of-function mouse experiments showed a positive correlation between sortilin expression and circulating PCSK9 levels. The positive correlation of serum sortilin and PCSK9 was supported in healthy human subjects and non–coronary artery disease (CAD) patients. The impact of sortilin on cholesterol homeostasis warrants further investigation.

Vascular Calcification
Vascular calcification contributes to and is an independent predictor of cardiovascular events. Computational modeling and clinical imaging studies suggest that subcellular microcalcifications in the fibrous cap of atherosclerotic plaque enhance the risk of plaque rupture. Our group recently elucidated the mechanisms by which sortilin is involved in cardiovascular calcification. We showed that calcifying extracellular vesicles released by vascular smooth muscle cells are the smallest nidi to form microcalcification. In mediating the formation of calcifying extracellular vesicles on molecular level, sortilin facilitates the trafficking of the calcification protein tissue-nonspecific alkaline phosphatase into extracellular vesicles, thus resulting in formation of vesicles with high mineralization competence. Tissue-nonspecific alkaline phosphatase activity is sufficient to drive vascular calcification but is also necessary for bone mineralization. Therefore, inhibition of vascular sortilin may reduce vascular tissue-nonspecific alkaline phosphatase activity and thereby vascular calcification. Post-translational modification of sortilin is one of the key mechanisms of vascular calcification. A C-terminal serine phosphorylation by Fam20C (extracellular serine/threonine protein kinase) or casein kinase 2 is essential for sortilin trafficking in calcifying smooth muscle cell. Therefore, targeting C-terminal serine phosphorylation may serve as a therapeutic strategy to reduce vascular calcification. In vitro and in vivo loss-of-function studies demonstrated a critical role of sortilin in the development of arterial calcification. Sortilin-deficient mice in a Ldlr-deficient background showed reduced vascular calcification without altering bone homeostasis. Bone marrow transplantation from sortilin-deficient mice to Ldlr-deficient mice demonstrated that vascular calcification was independent of infiltration of immune cells lacking sortilin. Importantly, our animal models did not show changes in cholesterol levels. We thus suggest that sortilin plays a direct role in ectopic calcification, independent of potential remote effects as a consequence of its lipid metabolism function.

Insulin Resistance
Different metabolic pathophysiological alterations, such as hyperglycemia, hyperinsulinemia, and insulin resistance, contribute to the initiation and progression of atherosclerotic plaques.

Sortilin promotes the biogenesis of insulin-responsive Glut4 (glucose transporter type 4) storage vesicles in adipocytes and myocytes, and impaired translocation of these vesicles is involved in the development of type II diabetes mellitus. Insulin resistance is a major cause of hepatic apolipoprotein apoB100/triglyceride overproduction in type II diabetes mellitus. Recent work suggests that sortilin plays a key role
in this pathway by altering hepatic apoB100 metabolism in insulin-resistant conditions.45

Further, under diet-induced obesity, sortilin-deficient mice gained less body weight and had enhanced glucose uptake in insulin tolerance tests.46 This favorable metabolic phenotype in liver and adipose tissue was in part mediated by reduced acid sphingomyelinase activity,46 an enzyme that may regulate ceramide levels, a major modulator of insulin signaling.27

Circulating Sortilin: Considering Sortilin as a Potential Cardiovascular Biomarker

Most studies have focused on modulating the expression of sortilin rather than exploring the effect of the soluble form. The main source of circulating sortilin that contributes to cardiovascular risk is not well understood. Experimental studies reported the ectodomain shedding of sortilin from neurons,48 tumor cells,49 and platelets.50 Sortilin can be shed by ADAM10.48 We and others demonstrated that sortilin can be packed into and released from extracellular vesicles,22,51 which could also contribute to circulating sortilin.

Only limited studies have assessed the circulating sortilin levels in patients with cardiovascular risk. The study by Japanese investigators was likely the first that revealed plasma sortilin levels in CAD patients demonstrating a 12±27% reduction by statin treatment during 8 months in 90 CAD patients.52 There is growing evidence that statins increase plaque stabilization by promoting coronary macrocalcification; however, the mechanism remains to be determined. We demonstrated that smooth muscle cell sortilin promotes plaque destabilizing microlcalfication; however, whether soluble sortilin has similar function and how statins affect microlcalfication formation are unexplored. Future studies may focus on the understanding of how statins affect sortilin secretion and whether statin use, increased sortilin secretion, and vascular calcification are causally linked. Of note, changes in total cholesterol and LDL-C levels did not correlate with sortilin levels, suggesting lipid-independent mechanisms.52 Plasma sortilin levels were higher in patients with elevated cardiovascular risk but without CAD history compared with CAD patients receiving aspirin therapy.50 Whether these results are an effect of the aspirin therapy or CAD history remains unclear, given that the authors demonstrated in vitro that aspirin suppresses sortilin release from activated platelets, and patients with increased cardiovascular risk but without CAD history had a higher platelet count. In addition, recent studies demonstrated an impact of aging on sortilin biology. Sortilin serum levels correlated negatively with age in men.22 In younger individuals, the presence of the minor (protective) allele of rs646776 associated with a greater genotype-specific difference in LDL-C levels than in older individuals.53 These data, however, need further confirmation. Another 2 studies showed higher sortilin levels in statin-naive CAD patients compared with non-CAD patients.40,54

We recently demonstrated a significant association of serum sortilin levels with both abdominal aortic calcification and cardiovascular events in a cohort of men aged >50 years (n=830).55 In multivariate-adjusted analysis, the third and fourth quartiles of sortilin associated with 3.4-fold and 3.8-fold higher risk of cardiovascular events compared with the first quartile. This association was independent of traditional Framingham risk factors, including LDL-C, C-reactive protein, and statin therapy.55 The abovementioned studies have considerable limitations, and well-designed larger studies are needed to confirm that serum sortilin levels may be a cardiovascular risk marker.

The role of sortilin-derived propeptide and its association with sortilin in cardiovascular disease are currently unknown. In a small cohort, Devader et al60 showed decreased propeptide serum levels in patients with major depressive disorder when compared with healthy controls. A mouse study supported this finding and suggested that sortilin-derived propeptide possesses antidepressive effects.57 Whether the circulating propeptide has a specific biological function and how it traffics from the late Golgi compartment and enters the blood stream remain largely unknown. The propeptide exhibits high affinity to mature sortilin and hinders ligand binding (eg, neurotensin).58 Determining whether the serum levels of soluble sortilin and sortilin-derived propeptide are physiologically interrelated requires further investigations.

Sortilin also exist as a variant (17b) that is extracellular released in a truncated form and may act as a decoy receptor.59 Sortilin 17b splicing variant is formed by the inclusion of exon 17b into SORT mRNA.59 Its regulation is reported in dementia.60 The contribution of soluble sortilin 17b to the circulating sortilin pool and the pathological consequences of abnormal splicing of sortilin in cardiovascular disease remain unknown.

There are several unanswered questions about sortilin biology that present an exciting research opportunity: What is the molecular mechanism contributing to release of circulating sortilin? Does soluble sortilin has a biological function (eg, receptor-mediated signaling, endocytosis) or is it only a surrogate marker? What is the relative contribution of soluble versus vesicle-packed sortilin to the circulation?

Sortilin Pathway: Possible Drug Target?
The multiple contributions of sortilin to cardiovascular risk suggest sortilin as a potential therapeutic target for cardiovascular disease. Hampering ligand binding to the Vps10 domain pocket by small molecules might be a feasible approach. Ligand binding has been shown to require furin-mediated propeptide cleavage.58 Neurotensin is a well-studied sortilin ligand that binds into the small binding pocket of the tunnel of the Vsp10 domain. Most ligand-sortilin binding cannot be blocked by neurotensin, suggesting that other ligands prefer different binding sites.23 Nevertheless, the reaction of small-molecule ligand AF40431 and its optimized successor AF38469 with the neurotensin-binding site of sortilin was recently reported.61,62 Whether the orally bioavailable AF38469 has a specific biological effect in vivo remains to be demonstrated.

A peptide library screen and mutation studies uncovered specific amino acid residues that are critical for selective pro-neurotensin interaction without affecting other receptor functions.63 However, the authors in this study suggested that this peptide is unlikely to be suitable for therapeutic use because of its low affinity.
Sortilin intracellular domain exerts its function via post-translational modification and ligand binding that impacts intracellular trafficking and sorting and thereby could serve as a drug target. We demonstrated by mutation studies that preventing sortilin phosphorylation at the C-terminal serine 825 reduces smooth muscle cell calcification.19 The phosphorylation at serine 825 determines the intracellular sortilin location to either the trans-Golgi network (when phosphorylated) or the lysosomal system (when not phosphorylated). A phosphoproteome screen showed additional potential phosphorylation at S19 and S821,44 but a functional consequence has not yet been reported. Li et al.65 identified a hepatic serine phosphorylation at 793 and 825 that is involved in dyslipidemia in type II diabetes mellitus.

An additional approach includes the modulation of sortilin expression via drug-based siRNAs, antibodies, or small molecules. Recent findings from a proteomic screen identified the small signal peptide-binding drug cyclotriazadisulfonamide, an anti-HIV agent, as an inhibitor of sortilin expression.66 To our knowledge, no small molecules targeting other parts of sortilin (tail or stalk) have been reported.

Sortilin-directed therapy to reduce atherosclerotic/metabolic risk might have adverse effects on the nervous system. Sortilin is essential for proper neuronal functionality by controlling the trafficking and release of neurotrophins and affect death signaling via p75NTR.67 However, recent evidence suggests sortilin as a risk factor for neurodegenerative diseases, including Alzheimer disease and frontotemporal dementia. Further understanding of the complex biology of sortilin in protein sorting and signaling may offer novel therapeutic strategies to combat cardiovascular and neuronal diseases.

Caution may be needed when using protein tags as an experimental tool in sortilin biology and in drug-screening systems to avoid methodological errors. Wild-type sortilin normally locates 90% inside the cell (ER/TGN [endoplasmatic reticulum/trans-Golgi network]/endosomes) and <10% at the cell surface.19 Adding tags to sortilin is likely to alter sortilin’s intracellular distribution and block GGA (ADP-ribosylation factor-binding protein)1/2/3 binding68 and consequently may alter pathways that are based on intracellular trafficking, endocytosis, and ligand binding.

To our knowledge, there are no available drugs that could target sortilin and disturb ligand binding to, for example, IL-6, ApoB, ApoE, or PCSK9. However, the authors think that sortilin could be a potential drug target. Because sortilin is a multiligand receptor, potential targeting strategies must be both tissue and pathway specific.

Concluding Remarks
There are several biological mechanisms through which sortilin may contribute to regulation of cardiovascular risk. For the development of cardiovascular therapeutic strategies targeting sortilin and its pathways, the multiple functions of sortilin must be considered. Outside the brain, sortilin is best known for its activities in lipid metabolism. Recently demonstrated effects of sortilin on vascular inflammation and calcification have, however, been shown to be independent of lipids. As sortilin is acting simultaneously at multiple levels, its global inhibition could have substantial effect on cardiovascular diseases.22,23 Future directions may include research on the pathways upstream of cellular sortilin regulation that is currently less understood. Gaining a better understanding on the biological function of soluble sortilin (eg, shedded, truncated, or vesicular) that may serve as a signaling molecule, and the regulatory impact of post-translational modification will broaden the options of sortilin’s druggability. In addition, although recent studies suggested a significant association of sortilin levels with both subclinical aortic atherosclerosis indices and cardiovascular events, larger clinical studies are needed to evaluate evidence for the clinical use of sortilin as a diagnostic or therapeutic tool.

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Disclosures
None.

References


**Highlights**

- Sortilin contributes to cardiovascular risk through several different biological mechanisms.
- Sortilin plays a role in atherosclerosis via arterial wall inflammation, calcification, and dysregulated lipoprotein metabolism.
- Circulating sortilin could serve as a cardiovascular risk biomarker.