Commentary on: From Noncoding Variant to Phenotype via \textit{SORT1} at the 1p13 Cholesterol Locus Musunuru et al. \textit{Nature}. 2010;466:714–719

and


Genome-wide association studies have the potential to lead to identification of novel pathways and mechanisms for the pathogenesis of common complex diseases. Previous genome-wide association studies have identified a locus on 1p13 as a risk locus for dyslipidemia and myocardial infarction. However, the responsible mechanisms have remained elusive. Two recent reports implicate \textit{SORT1} as the causative gene underlying a human chromosome 1 LDL/coronary artery disease locus but come to opposite conclusions concerning \textit{SORT1}’s role in the regulation of VLDL secretion.

Human genome wide association studies have had spectacular success in identifying single nucleotide polymorphisms (SNPs) in or near genes associated with variation in plasma lipid and lipoprotein levels. More than 100 loci have been found that account for a significant part of the genetic variation in triglyceride, low-density lipoprotein (LDL), and high-density lipoprotein cholesterol levels. Many of these loci involve novel genes or regions. Genome-wide association studies of coronary artery disease or myocardial infarction have identified a smaller number of genetic loci, some of which are also associated with changes in traditional lipoprotein risk factors. A notable example has been a widely replicated chromosome 1p13 locus that has been associated with both myocardial infarction and LDL cholesterol levels and that does not contain a gene known to give rise to a major alleles at this locus are present in about 65% to 80% of homozygosity for the minor alleles, are associated with a 20% to 40% increase in the risk of myocardial infarction and up to 16 mg/dL higher LDL cholesterol levels. The relevant SNPs are localized in a gene cluster containing 4 genes, \textit{CELSR2}, \textit{PSRC1}, \textit{MYBPHL}, and \textit{SORT1}. A recent study has shown that the SNPs most strongly associated with LDL levels are localized to an intergenic region between \textit{CELSR2} and \textit{PSRC1} and that the minor allele SNP with strongest association creates a functional C/EBPalpha binding site. The authors provide evidence that this leads to an increase in the expression of \textit{SORT1} in human hepatocytes. Studies using forced over- or underexpression in mice demonstrate that higher \textit{SORT1} levels are associated with reduced hepatic very low-density lipoprotein (VLDL) secretion and lower LDL cholesterol levels, matching findings that lower levels of hepatic \textit{SORT1} messenger RNA (mRNA) are associated with higher levels of LDL cholesterol in humans.

\textit{SORT1} is one of five members of a Vps10p domain receptor family, often found in the \textit{trans}-Golgi network and early endosomes. The VPS10 domain is a 10-bladed beta-propeller. \textit{SORT1} is made as a proprotein that is cleaved in the Golgi by proprotein convertases, allowing it to bind ligand. \textit{SORT1} appears to be a multiligand receptor and has been reported to bind LPL and ApoAV. \textit{SORT1} is involved in the formation and insulin-responsiveness of GLUT-4 storage vesicles during adipocyte differentiation. In the brain, \textit{SORT1} may form part of a signaling complex that regulates cell survival. The in vivo relevance of these multifaceted properties remains uncertain.

In a tour de force of genetic, biochemical, and animal experiments, Musunuru et al implicate \textit{SORT1} as the causative gene at the 1p13 locus. While genetic fine mapping led to the identification of 6 SNPs with similar lowest \textit{P} values for association with LDL cholesterol in white populations, evaluation of the same SNPs in African-Americans revealed a single SNP associated with the lowest \textit{P} values. When a 6-Kbp DNA element representing a haplotype bloc containing the minor alleles was placed in front of the luciferase gene, there was higher activity compared with the element containing the major alleles. Strikingly, when each variant SNP was changed from the minor to the major haplotype version, only the SNP with strongest association in African-Americans led to the expected decrease in luciferase activity. This also led to the loss of C/EBPalpha binding and activity. These findings strongly suggest that Musunuru et al have identified the causative SNP that changes a G to a T in an enhancer-like element located between the \textit{CELSR2} and \textit{PSRC1} genes. They also show that the minor allele is associated with more than 12-fold higher levels of \textit{SORT1} mRNA, 12-fold higher levels of \textit{PSRC1} mRNA, and no significant change for \textit{CELSR2} mRNA levels in the liver. Notably, however, there was no differential expression of any of these genes in adipose tissue. The authors provide limited evidence that the minor allele also results in higher induction of \textit{SORT1} in response to transfection of \textit{CEBPalpha} using a human stem cell model partially differentiated toward hepatocytes.

In a separate report, Kjolby et al also focus on \textit{Sort1} as a candidate gene and implicate \textit{Sort1} in the regulation of VLDL secretion and LDL cholesterol levels, using \textit{Sort1} knockout mice and \textit{Sort1} overexpression in mouse models and cultured hepatocytes. In contrast to the conclusions of Musunuru,
however, they conclude that that decreased Sort1 expression is associated with lower levels of VLDL secretion and LDL cholesterol, with Sort1 overexpression producing the opposite result. These authors provide convincing evidence that SORTILIN-1 is associated with ApoB in the Golgi of hepatocytes and show that SORTILIN-1 binds ApoB100 but not ApoB48.

While searching for a possible explanation for these disparate findings, we noted that no experiment performed by either group is done using exactly the same models or conditions. Most of the studies by Musunuru et al are done in chow-fed ApoBTg Apobec1-/- mice using AAV8 vector to achieve overexpression or duplex siRNA delivered in a liposomal-like preparation for 70% to 80% knockdown. In contrast, Kjolby et al used Sort1-/- mice or overexpressed Sort1 using an adenovirus in Western-type diet fed mice on Ldlr-/- or wild-type backgrounds. In the studies by Musunuru et al, the effects on LDL cholesterol were more pronounced in the ApoBTg Apobec1-/- model (−120% for knockdown, −73% for overexpression, +120% for knockdown) than in the Ldlr-/- Apobec1-/- mice (−29% AAVSort1, +16% to 22% siRNA). The latter result contrasts with a 25% decrease for LDL cholesterol in Sort1-/- mice (in wild-type or Ldlr-/- background). Thus, in the Ldlr-/- background, the results of knockout and knockdown are only moderate in both groups, though clearly in the opposite direction. These could be real differences. It is possible that in the context of marked overexpression of ApoB, SORTILIN-1 has a predominant role in mediating degradation, whereas at lower expression levels of ApoB it does not, or that a low basal level could even be required for normal secretion as suggested by the Sort1-/- mice.

While the interventions used by Musunuru et al were liver specific, the whole-body Sort1 knockout likely results in reduced Sort1 in other tissues, such as adipose as well as in liver. Thus, there could be confounding effects of higher LPL activity in adipose (decreased degradation) resulting in lower VLDL cholesterol and triglyceride levels. Sort1 adenosine could perhaps also be expressed in adipose and lead to an increase in LPL degradation. The Kjolby et al data are somewhat unconvinced with respect to providing evidence that VLDL secretion is decreased in knockout mice in vivo. They show only small decreases in triglyceride secretion and no change in ApoB and cholesterol secretion.

Perhaps the most directly contradictory result is that obtained using Sort1 knockouts, in which increased LDL and total cholesterol was reported by Musunuru et al, in contrast to the decrease reported by Kjolby et al. However, again there are important differences, such as different diets (Western-type diet vs chow). Moreover, these are different knockouts. The Sort1 knockout mouse that Musunuru et al used was made by replacing a segment between exons 2 and intron 3 of the Sort1 gene with the neomycin-resistance cassette, whereas the mice used by Kjolby et al were made by replacement of a fragment of 126 base pairs from exon 14 and 303 base pairs of the subsequent intron sequence of Sort1 with Neo. It seems that the Sort1 knockout used by Kjolby et al, which only removed exon 14 from the gene, was associated with low intracellular expression of a truncated protein. It is theoretically possible that such a protein could have residual activity to mediate VLDL degradation.

Although Musunuru et al show increased SORT1 mRNA in the liver for carriers of the minor allele, as pointed out by Kjolby et al, this does not necessarily indicate that SORTILIN-1 protein levels are similarly changed. Indeed, data shown on SORTILIN-1 protein levels in liver were very limited. Also, a role for other genes in the locus, such as PSRC1 or CELSR2, could not be completely excluded, and they could perhaps have effects opposite to SORTILIN-1 on VLDL secretion. However, Musunuru et al reported that overexpression of PSRC1 did not affect LDL cholesterol levels. Overall, both groups have made valuable contributions that indicate a key role of SORT1 in explaining the effects of the chromosome 1p13 locus on LDL cholesterol and coronary artery disease. The Musunuru et al data provide a more satisfying explanation of the human findings in which higher levels of hepatic SORT1 mRNA are associated with lower LDL cholesterol levels, but the conflicting results regarding VLDL secretion still need to be resolved.

What lies ahead? An exchange of reagents between different laboratories with assessment of lipoprotein changes under the exact same experimental conditions may help to resolve some of the discrepancies. Studies using tissue-specific knockouts may be revealing. Finally, focused assessment by point mutagenesis of the putative causative SNP on SORT1 gene expression and VLDL secretion using human hepatocytes differentiated from ES or iPSC cells would be desirable. These issues will have to be sorted out before SORTILIN-1 can become a well-validated target for therapy.

References


Sorting Out Sortilin
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