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Commentary

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ASGR1 and cholesterol: connecting the dots

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There is abundant evidence in support of the hypothesis that non-HDL/LDL cholesterol contributes directly to the development of atherosclerotic cardiovascular disease (ASCVD) in humans[1]. Further, both genetic and clinical trial data show a linear correlation between the lowering of non-HDL cholesterol and reduction in ASCVD - independent of the cholesterol-lowering mechanism[1]. While concerns have been raised that extreme cholesterol lowering may adversely impact health, mutations that cause lifelong very low non-HDL cholesterol have not been associated with serious adverse effects[2]. In fact, no level of LDL cholesterol below which benefit ceases or harm occurs has been defined[3]. The importance of lowering non-HDL/LDL cholesterol to prevent and treat ASCVD thus cannot be overstated and is reflected in various guidelines.

Despite all of the above, in a recent study, less than half of ASCVD patients were found to be treated according to the ACC/AHA blood cholesterol treatment guidelines[4]. While the reason for this is no doubt multifactorial, individual variability in response to cholesterol-lowering drug therapy and increased emphasis on a tailored approach to management calls for additional drugs with novel mechanisms of action.

In 2016, in a study by deCODE genetics, rare loss-of-function variants in the gene *ASGR1*, disrupting the function of the protein, were found to be associated with lower levels of non-HDL cholesterol and a lower risk of coronary artery disease[5]. The mechanism mediating the cholesterol-lowering effect of ASGR1 was not identified. ASGR1 is the major unit of the asialoglycoprotein receptor, a liver-specific transmembrane protein that mediates the endocytosis and lysosomal degradation of desialylated glycoproteins following

binding to terminal galactose or N-acetylgalactosamine groups. Importantly, the asialoglycoprotein receptor, which is present in abundance on hepatocytes, is a vehicle of major interest for liver-specific drug delivery that is, for example, currently used for uptake of inclisiran, a small interfering RNA against PCSK9 for lowering of cholesterol.

In an article published in Nature in August 2022, Wang *et al.* provided evidence through a series of experiments for how the inhibition or deletion of ASGR1 leads to lower lipid levels through the promotion of cholesterol excretion[6]. The authors generated *Asgr1*-deficient mice which not only had lower total cholesterol in serum and liver than wild-type mice, but also increased concentrations of biliary cholesterol and bile acids in the gallbladder. The mice had drastically increased liver levels of the protein LXRα, a nuclear receptor with a major role in lipid metabolism, and a consistent increase in the expression of *Abca1*, *Abcg5/8* and *Cyp7a1*, which are regulated by LXRα. These genes all encode proteins involved in cholesterol and bile acid homeostasis that could explain the lipid changes noted in the mice; ABCG5/G8 promotes biliary cholesterol excretion, ABCA1 transports cholesterol to apolipoprotein A-I to form HDL particles, and CYP7A1 is the rate-limiting enzyme in bile acid synthesizes from cholesterol. However, protein levels of ABCG5/8, ABCA1 and CYP7A1 were not increased in mice deficient in both *Asgr1* and *Lxrα*, confirming the central role of LXRα in mediating the effect of ASGR1 in mice. Interestingly, despite the elevation of LXRα in *Asgr1* deficient mice, levels of SREBP1, a key player in the induction of lipogenesis by the liver that is also regulated by LXRα, were not increased. Further, experiments by the authors using cell lines were consistent with the idea that ASGR1 deficiency inhibits lysosomal mTORC1 and activates AMPK in the absence of sugars and amino acids otherwise released from lysosomal degradation. mTORC1 and AMPK are protein complexes with major roles in regulating cellular energy and metabolism. Inhibition of mTORC1 and activation of AMPK led to increased LXRα but also to inhibition of SREBP1 and suppressed lipogenesis, potentially explaining the lack of increase in SREBP1 in the *Asgr1*-deficient mice. This last part is important because while the therapeutic potential of LXRα for a range of disorders is well recognized, systemic activation of LXR is complicated by hepatic lipogenesis, steatosis and hypertriglyceridemia due to induction of SREBP1. It is also worth mentioning that the authors did not observe changes in LDL uptake or levels of LDLR in *Asgr1*-deficient mice, as others have reported.

The authors also reported results from several in-vivo animal experiments supporting their findings. The reaction to asialofetuin A, a natural ligand for ASGR1, in wild-type mice, was consistent with activation of mTORC1, inhibition of AMPK, and the downstream effects of increased total cholesterol and decreased excretion of cholesterol; in contrast, *Asgr1*-deficient mice had suppressed mTORC1, activated AMPK, and a better metabolic profile, none affected by asialofetuin A. They also used the mTOR inhibitor rapamycin to mimic the *Asgr1*-deficiency-induced mTORC1 inhibition and confirmed that inhibiting mTORC1 activates AMPK with the anticipated outcomes. Silencing subunits of AMPK with an RNA inhibitor confirmed the role of AMPK in the model, and stimulation of LXR and AMPK with respective agonists is consistent with AMPK activation inhibiting lipid biosynthesis and distinguishes ASGR1 depletion from direct LXR activation.

With this, the authors have arrived at a plausible explanation of how ASGR1 deficiency lowers non-HDL cholesterol: ASGR1 deficiency reduces endocytosis and lysosomal degradation of glycoproteins and thus inhibits mTORC1 and activates AMPK; this, in turn, upregulates LXRα, ABCA1, ABCG5/8 and CYP7A1, promoting cholesterol excretion, without induction of lipogenesis.

To test their hypothesis, Wang *et al.* silenced *Asgr1* in mice with an RNA inhibitor and observed significantly increased protein levels of LXRα, ABCG8, ABCA1 and CYP7A1, decreased *Srebp1c* messenger

RNA, decreased total cholesterol in serum and liver and increased biliary cholesterol and bile acids[6]. Injection of monoclonal anti-ASGR1 neutralizing antibodies produced similar findings, supporting their model. Additionally, silencing *Asgr1* prevented atherosclerotic plaque formation in *Ldlr*-deficient mice on an atherosclerosis-inducing diet suggesting that the effect of the ASGR1 inhibition/deletion is independent of the LDLR. Finally, the authors observed synergistic lipid-lowering effects in mice between the monoclonal antibody and both atorvastatin and ezetimibe.

The substantial body of work by Wang *et al.* suggests that the inhibition of ASGR1 lowers cholesterol in the blood through cholesterol excretion, a mechanism that is currently not exploited by any of the three major classes of cholesterol-lowering drugs now available[6]. Statins, competitive inhibitors of the enzyme HMG-CoA reductase, reduce cholesterol synthesis in the liver and increase hepatic uptake of LDL from the blood through upregulation of the LDL receptor; ezetimibe inhibits intestinal uptake of dietary and biliary cholesterol by interacting with NPC1L1, which leads to upregulated *LDLR* expression and increased clearance of LDL from the blood; and PCSK9 inhibitors lower circulating LDL-C levels by stabilizing LDLR. This complementary mechanism with potentially synergistic action with other available therapeutics makes ASGR1 an attractive additional therapeutic possibility for lowering non-HDL/LDL cholesterol and reducing residual ASCVD. Another obvious advantage of the asialoglycoprotein receptor as a potential drug target is the several feasible options for targeting this liver-specific receptor, including inhibitory antibodies, silencing RNA, and antisense oligonucleotides.

In the original study by deCODE genetics that linked loss-of-function variants in *ASGR1* to lower non-HDL/LDL cholesterol and reduced risk of coronary artery disease, the same variants were also associated with increased levels of vitamin B12 and alkaline phosphatase[5]. While this raises concerns of liver damage due to loss-of-function of ASGR1, no other measures of liver function were found to be abnormal in that study and the authors proposed that the likely explanation for the increased alkaline phosphatase levels was compromised clearance of desialylated molecules from the circulation. Still, a concern for the risk of gallstones due to increased cholesterol content in bile remains, and indeed, variants in *ABCG5/8* that associate with lower non-HDL/LDL cholesterol also associate with an increased risk of gallstones[7]. However, no disease associations have been reported for *ASGR1* in humans that would suggest negative effects of inhibiting ASGR1 and, further, Icelandic carriers of the del12 *ASGR1* loss-of-function variant lived 1.5 years longer than non-carriers. Mice lacking *Asgr1* have also been found to thrive normally. However, the full consequences of inhibiting the ASGR1 receptor, including potential serious adverse effects, are not completely understood.

To summarize, the experiments of Wang *et al.* are consistent with the loss of function of ASGR1 lowering non-HDL/LDL cholesterol through cholesterol excretion, a mechanism that is not utilized by existing cholesterol-lowering drugs[6]. No serious adverse effects of ASGR1 inhibition have been identified and carriers of *ASGR1* loss of function variants live longer than non-carriers, making ASGR1 an attractive target for lowering cholesterol and reducing the risk of ASCVD.

DECLARATIONS

Authors' contributions

The author contributed solely to the article.

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Conflicts of interests
Holm H. is an employee of deCODE genetics/Amgen Inc.

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Not applicable.

Consent for publication
Not applicable.

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