

Not just correlative: a new pathway defines how an *ALDH2* SNP contributes to atherosclerosis

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Individuals with the rs671 SNP in the gene encoding aldehyde dehydrogenase 2 (*ALDH2*) are at increased risk of cardiovascular disease (CVD); however, it has been unclear if this mutation contributes to CVD development. In this issue of the *JCI*, Zhong et al. perform an elegant set of experiments that reveal a pathway wherein the *ALDH2* rs671 mutant is phosphorylated by AMPK and translocates to the nucleus where it represses the transcription of a lysosomal H⁺ pump subunit that is critical for lipid degradation and foam cell formation, as occurs in atherosclerosis. The discovery of this pathway may explain how subjects harboring *ALDH2* rs671 are at a greater risk for numerous other disease states and thereby provide new targets for therapeutic intervention.

Genetic risk factors for atherosclerosis

Atherosclerosis is the leading cause of cardiovascular disease (CVD) and the primary determinant of heart disease and stroke, the top two killers worldwide (1). The high mortality rate associated with atherosclerosis is of particular importance for individuals suffering from familial hypercholesterolemia, a genetic disorder whereby the loss of at least one functional LDL receptor (*LDLR*) allele results in the inefficient clearance of circulating cholesterol and aggressive atherosclerotic plaque formation resulting in premature CVD (2, 3). Another genetic determinant that predisposes individuals to CVD is a SNP in the gene encoding the alcohol-metabolizing enzyme aldehyde dehydrogenase 2 (*ALDH2*). Approximately 40% of East Asians carry this SNP (*ALDH2* rs671), which is associated with the phenomenon known as “Asian flush” that occurs with alcohol consumption. While *ALDH2* rs671 is associated with an increased risk of atherosclerosis and coronary artery disease (4), it is unknown

whether this SNP causally contributes to CVD development.

ALDH2 and atherosclerosis development

In this issue, Zhong et al. provide important mechanistic evidence to address the role of *ALDH2* rs671 in CVD (5). The authors employed mouse genetics and deleted *Aldh2* in the *Ldlr* knockout background (*Ldlr*^{-/-}), a commonly employed experimental model of atherosclerosis, and demonstrated that loss of *ALDH2* reduced plaque burden and foam cell formation (fat laden macrophages that contribute to plaque generation and growth). This unexpected observation suggested a critical role for *ALDH2* in atherosclerosis pathogenesis. Bone marrow-derived macrophages were identified as the primary cell type responsible for the reduction in plaque load, as transplantation of bone marrow from *Aldh2*^{-/-} *Ldlr*^{-/-} mice into *Ldlr*^{-/-} mice was sufficient to prevent atherosclerotic plaque formation. As macrophage-mediated clearance of circulating lipids is a key contributor to ath-

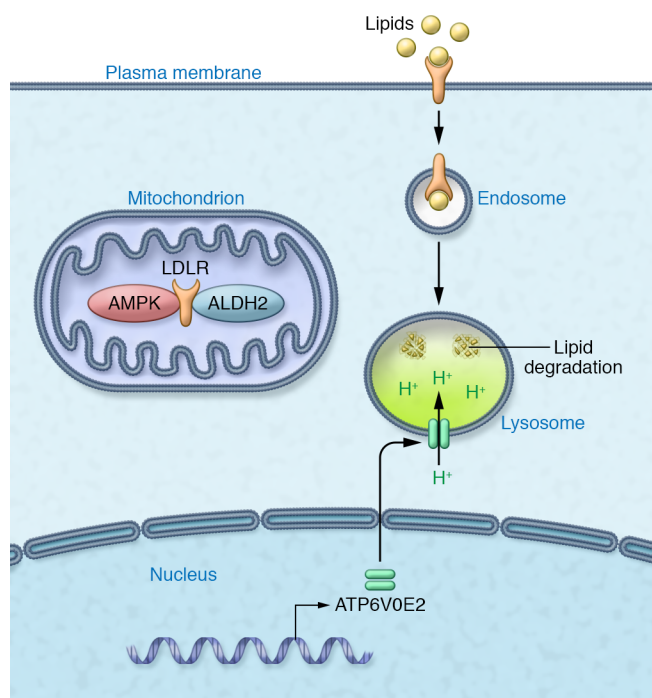
erosclerotic progression (6), Zhong and colleagues next interrogated macrophage foam cell formation and the capacity of these cells to metabolize oxidized lipids (uptake, degradation, and efflux). Strikingly, *Aldh2*^{-/-} *Ldlr*^{-/-} macrophages possessed an enhanced lysosomal cholesterol degradation capacity, which correlated with a reduction in foam cell formation. Importantly, *Aldh2* deletion alone did not alter lysosomal degradation, indicating that the phenotype was *LDLR*-dependent. In a carefully designed set of experiments, the authors determined that autolysosome number was substantially increased in *Aldh2*^{-/-} *Ldlr*^{-/-} macrophages, suggesting an enhanced ability to clear lipid-laden endosomes. Notably, this protective effect was abolished when lysosomal pH was neutralized with bafilomycin A1, providing mechanistic links between *ALDH2*, lysosomal function, and foam cell formation. Collectively, these results suggested the contribution of a pathway that is dependent on *ALDH2* and *LDLR* to the progression of atherosclerosis.

Next, Zhong et al. explored the underlying mechanism by which the interaction of *ALDH2* and *LDLR* regulates foam cell formation and examined *ALDH2* enzymatic activity, which was not different between WT and *Ldlr*^{-/-} mice (5). This result suggested the potential for a direct interaction between the two proteins, and an interaction between *LDLR* and *ALDH2* was experimentally confirmed by coimmunoprecipitation. The authors hypothesized that the interaction of *LDLR* and *ALDH2* prevented nuclear translocation, as observed in *Ldlr*^{-/-} macrophages, and that this may be in part mediated by AMPK phosphorylation. This postulate was supported by previous work showing that AMPK regulates *ALDH2* translocation to the nucleus, where it can act as a transcriptional repressor (7). Using transcriptomic RNA-Seq analysis in *Aldh2*^{-/-} *Ldlr*^{-/-} and *Ldlr*^{-/-} macrophages, the authors identified several genes that were regulated by

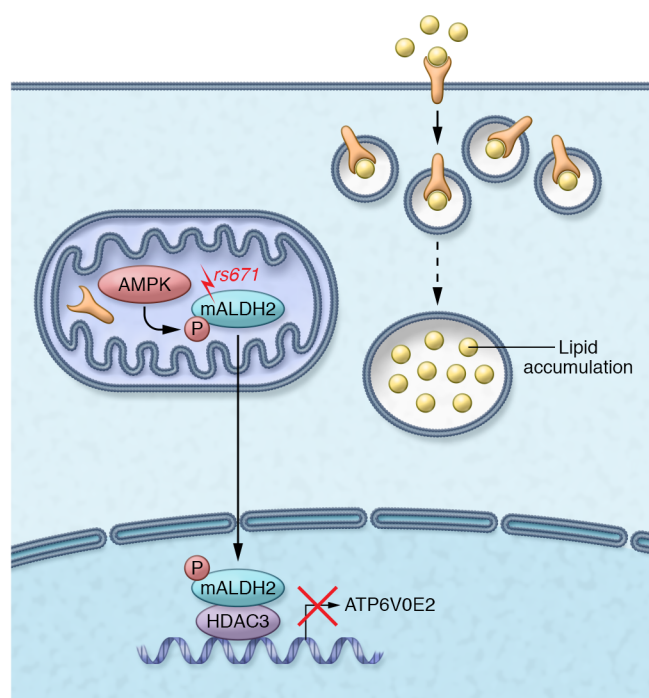
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A Normal macrophage clearance of lipids

Efficient lysosomal lipid degradation

B *ALDH2* rs671 SNP-mediated foam cell formation

Lipid accumulation and foam cell formation

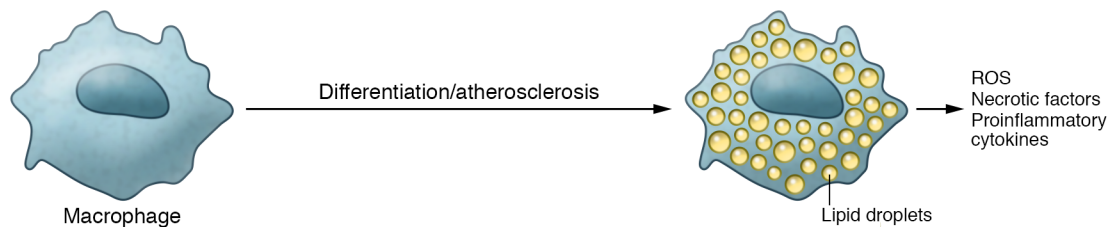


Figure 1. An *ALDH2* SNP contributes to atherosclerosis. In this issue of the *JCI*, Zhong et al. (5) identify a pathway that reveals how the *ALDH2* rs671 SNP promotes atherosclerosis. **(A)** The low-density lipoprotein receptor (LDLR) physically interacts with aldehyde dehydrogenase 2 (ALDH2) and 5' AMP-activated protein kinase (AMPK) to prevent AMPK-mediated phosphorylation and subsequent nuclear translocation of ALDH2. **(B)** The *ALDH2* mutant protein (mALDH2 rs671 SNP) does not interact with LDLR, thereby removing the break on AMPK-mediated phosphorylation. Phospho-mALDH2 translocates to the nucleus and in an HDAC3-dependent fashion suppresses transcription of the lysosomal H^+ pump subunit, ATP6V0E2. ATP6V0E2 is critical for lysosomal acidification and autophagic clearance of lipids. The loss of this critical pathway accelerates foam cell formation and atherosclerosis.

ALDH2. Of particular interest, Atp6v0e2, a lysosomal proton pump that is required for proper lysosomal function and lipid clearance, was the third most upregulated gene. In a series of elegant molecular studies, Zhong et al. revealed that AMPK phosphorylation of *ALDH2* promotes translocation to the nucleus, where its presence suppressed *ATP6v0e2* transcription, all of which was prevented when LDLR was available to directly bind and sequester AMPK and *ALDH2*, preventing phosphorylation of *ALDH2* and its nuclear translocation (Figure 1). This finding was important, as it provides a molecular mechanism that accounts for the authors' previous

observations of lysosomal dysfunction mediating enhanced foam cell formation.

Conclusions and clinical implications

This serendipitous discovery was further extended toward clinical relevance, as expression of mutant *ALDH2* rs671 mimicked the signaling observed with loss of LDLR. Moreover, the *ALDH2* SNP prevented the direct interaction with LDLR and allowed for enhanced AMPK interaction and phosphorylation, resulting in *ALDH2* rs671 nuclear translocation. This mechanism, in the context of earlier results, provides an explanation for the

predisposition of this patient population to develop atherosclerosis and coronary heart disease. Convincingly, macrophages differentiated from peripheral blood monocytes isolated from human subjects carrying the *ALDH2* rs671 SNP displayed enhanced nuclear localization of *ALDH2*, diminished ATP6V0E2 lysosomal expression, and impaired cholesteryl ester hydrolysis. Furthermore, Zhong et al. showed that activation of AMPK by metformin treatment enhanced *ALDH2* translocation and foam cell formation in human macrophages containing the *ALDH2* rs671 SNP. This finding has significant clinical implications, as metformin is a regularly

prescribed medication for individuals with diabetes, a comorbidity often associated with atherosclerosis. To this point, a recent meta-analysis indicated that the *ALDH2* rs671 SNP also correlated with the incidence of type 2 diabetes (8); therefore, prescription of metformin may further increase the risk of atherosclerosis in patients with the *ALDH2* rs671 SNP and perhaps this novel signaling pathway is also linked to the pathogenesis of diabetes.

While the study by Zhong et al. provides causative evidence linking *ALDH2* to atherosclerosis (5), the exact role of *ALDH2* in disease progression remains to be elucidated. There are several questions that have been brought to light by the current study and require further investigation to provide a more holistic view. First, what are the signaling events that lead to AMPK activation during atherosclerosis pathogenesis? As Zhong et al. mention, this appears to not be due to augmentation of conventional regulators (e.g., gene transcription, AMP/ATP levels). A better understanding of the upstream signaling events that lead to AMPK activation and *ALDH2* phosphorylation could provide crucial mechanistic insight and novel therapeutic targets for *ALDH2* SNP carriers. Second, where does the *ALDH2*-LDLR-AMPK interaction take place and what determines the subcellular localization of this interaction? Specifically, how do LDLR and AMPK enter the mitochondria to physically interact with *ALDH2* and how does this modulate LDLR cycling that occurs with lipid uptake? These fundamental questions need to be addressed to place this novel signaling within the canonical LDLR lipid uptake pathway. Third, how is phosphorylated *ALDH2* transported out of the mitochondria and into the nucleus and how is AMPK-mediated phosphorylation regulated in the mitochondrial matrix environment? There are limited data to explain how AMPK may function in the mitochondria where the pH is significantly different than the cytosol,

and what is the phosphatase counteracting AMPK phosphorylation. Fourth, what other genes, beyond *ATP6V0E2*, does *ALDH2* regulate and what is the impact on macrophage polarization, inflammation, and secretory factors. Further identification of the genes modulated by *ALDH2* nuclear translocation and their impact on the vascular microenvironment could provide additional targets for translational application. Fifth, does a similar mechanism in the regulation of cholesterol metabolism exist outside of the macrophage? Hepatocyte clearance of LDL is the greatest contributor to circulating cholesterol levels. Does this pathway play a role in global lipid metabolism? Sixth, are patients with type 2 diabetes and the *ALDH2* SNP who are currently taking metformin at a greater risk of progressive atherosclerosis? Based on the results of Zhong et al., this appears to be an area in need of critical and immediate attention. Finally, what is the functional consequence of this novel *ALDH2* signaling pathway in other disease states? Looking beyond atherosclerosis, it is interesting to note that *ALDH2* rs671 is also associated with an increased risk of hypertension (9), diabetes mellitus (8), osteoporosis (10), and cancers (11, 12). Does nuclear translocation of *ALDH2* contribute to the pathogenesis of these other disease states?

In summary, the current study by Zhong et al. (5) makes an important mechanistic link regarding the increased prevalence of atherosclerosis in individuals with the *ALDH2* rs671 SNP. While the complete underlying mechanisms remain to be elucidated, these findings provide the framework to further delineate the involvement of *ALDH2* in not only atherosclerosis, but numerous other disease states of clinical significance.

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1. Benjamin EJ, et al. Heart disease and stroke statistics-2018 update: a report from the American Heart Association. *Circulation*. 2018;137(12):e67–e492.
2. Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, Wierzbicki AS. Familial hypercholesterolaemia. *Nat Rev Dis Primers*. 2017;3:17093.
3. Ishibashi S, Brown MS, Goldstein JL, Gerard RD, Hammer RE, Herz J. Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. *J Clin Invest*. 1993;92(2):883–893.
4. Han H, Wang H, Yin Z, Jiang H, Fang M, Han J. Association of genetic polymorphisms in ADH and ALDH2 with risk of coronary artery disease and myocardial infarction: a meta-analysis. *Gene*. 2013;526(2):134–141.
5. Zhong S, et al. Acetaldehyde dehydrogenase 2 interactions with LDLR and AMPK regulate foam cell formation. *J Clin Invest*. 2019;129(1):252–267.
6. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 2011;145(3):341–355.
7. Choi JW, et al. Malondialdehyde inhibits an AMPK-mediated nuclear translocation and repression activity of ALDH2 in transcription. *Biochem Biophys Res Commun*. 2011;404(1):400–406.
8. Li GY, et al. Meta-analysis on the association of ALDH2 polymorphisms and type 2 diabetic mellitus, diabetic retinopathy. *Int J Environ Res Public Health*. 2017;14(2):E165.
9. Hasi T, Hao L, Yang L, Su XL. Acetaldehyde dehydrogenase 2 SNP rs671 and susceptibility to essential hypertension in Mongolians: a case control study. *Genet Mol Res*. 2011;10(1):537–543.
10. Hoshi H, et al. Aldehyde-stress resulting from Aldh2 mutation promotes osteoporosis due to impaired osteoblastogenesis. *J Bone Miner Res*. 2012;27(9):2015–2023.
11. Jiang Y, Zhang J, Wu Y, Wang J, Li L. Association between ALDH2 rs671 G>A polymorphism and gastric cancer susceptibility in Eastern Asia. *Oncotarget*. 2017;8(60):102401–102412.
12. Park JY, et al. Impact of smoking on lung cancer risk is stronger in those with the homozygous aldehyde dehydrogenase 2 null allele in a Japanese population. *Carcinogenesis*. 2010;31(4):660–665.