ATP-Binding Cassette Transporter A1 in Lipoprotein Metabolism and Atherosclerosis
A New Piece of the Complex Puzzle

Miranda Van Eck, Theo J. C. Van Berkel

ATP-binding cassette transporter A1 (ABCA1) is a key protein determining high-density lipoprotein (HDL) function. In 1999, it was discovered by 3 independent groups that mutations in the gene for ABCA1 underlie the molecular defect in the HDL deficiency syndrome Tangier disease. Subsequent studies with genetically engineered mice lacking or overexpressing ABCA1 provided evidence that ABCA1 modulates atherosclerosis susceptibility on either end of the reverse cholesterol transport pathway. In the liver (and to a lesser extent in intestine), it determines the biogenesis of nascent HDL particles, whereas in macrophages it is essential for the prevention of the excess cholesterol accumulation by facilitating the transport of cellular cholesterol and phospholipid onto lipid-poor apo AI, the major apoprotein of HDL.

Surprisingly, the first studies by Aiello et al investigating the effects of ABCA1 deletion in all endogenous tissues failed to show any affect on atherosclerosis susceptibility, neither in the apolipoprotein E knockout (apoE KO) background nor in the low-density lipoprotein receptor knockout (LDLr KO) background. Both models, however, did show marked decreases in proatherogenic apoB lipoproteins on deletion of ABCA1. One could thus also argue that total body ABCA1 KO mice still developed substantial atherosclerosis despite the markedly decreased levels of proatherogenic lipoproteins. Total body KO mice also lack ABCA1 in bone marrow–derived cells, including macrophages that accumulate in the arterial wall during atherosclerotic lesion development. Several bone marrow transplantation studies were performed in LDLr KO and apoE KO mice to investigate the effects of specific deletion of ABCA1 in bone marrow–derived cells. Deletion of ABCA1 in bone marrow–derived cells was also associated with decreased levels of apoB lipoproteins, a phenotype that became even more pronounced on combined deletion of ABCA1 with ABCG1, another transporter controlling cellular cholesterol efflux. Importantly, despite the lower levels of proatherogenic apoB lipoproteins, specific disruption of ABCA1 in bone marrow–derived cells induced atherosclerotic lesion development. It is thus likely that the antiatherogenic effects of the reduced apoB lipoproteins in the total body ABCA1 KO mice were compensated by the proatherogenic effects of ABCA1 deletion in bone marrow–derived cells.

The tissue-specific effects of ABCA1 on atherosclerosis susceptibility were dissected out further by the groups of Parks and Hayden using ABCA1 floxed mice that were crossed with mice expressing the Cre recombinase transgene under control of tissue-specific promoters. Surprisingly and in contrast to the findings of the bone marrow transplantation studies, myeloid-specific ABCA1/LDLr KO mice, generated by crossing the ABCA1 floxed mice with animals expressing Cre recombinase under control of the LysM promoter, did not show an increased susceptibility to atherosclerosis. Lymphoid-specific ABCA1 KO mice are currently highly awaited to find out whether the proatherogenic effects of ABCA1 deletion in bone marrow–derived cells are attributable to deleterious effects on B and T lymphocytes. Liver-specific effects of ABCA1 deletion were investigated by crossbreeding of ABCA1 floxed mice with mice expressing Cre recombinase under control of the albumin promoter. ApoE KO mice lacking ABCA1 specifically in liver (HSKO), developed significantly larger atherosclerotic lesions than control apoE KO mice after 12 weeks on chow diet, suggesting that hepatic ABCA1 is antiatherogenic. In this issue Bi et al, however, show that hepatocyte-specific deletion of ABCA1 in the LDLr KO background does not affect the development of early atherosclerotic lesions after 5 weeks challenge with a Western-type diet containing 10% palm oil and 0.2% cholesterol. Interestingly, after 16 weeks challenge with the atherogenic diet, a striking reduction in lesion development was observed in the aortic root, both in male and female LDLr KO mice. In face lesion analysis in the aorta also showed a tendency to reduced atherosclerosis in females after 16 weeks atherogenic diet challenge, but not in males. For both sexes, however, a trend toward a lower cholesterol content was found in the aorta, indicative of reduced lesion development. Liver-specific deletion of ABCA1 thus (paradoxically) seems to reduce the development of advanced lesions in LDLr KO mice.

Interestingly, when the cholesterol content in the aortas of both the current study and the study in apoE KO mice, published by Brunham et al, is plotted against the effect on lesion development, it is clear that hepatic ABCA1 deletion is
more atheroprotective when the accumulation of cholesterol in the aorta is higher and thus lesions are larger and likely more advanced (Figure). Unfortunately, neither in the previous apoE KO/HSKO study nor in the current LDLr KO/HSKO study a thorough morphometric analysis of the lesions was performed, making it impossible to discriminate whether it is the actual cholesterol content in the aorta or the stage of lesion development that determines whether hepatic ABCA1 deletion is pro- or antiatherogenic.

Macrophage foam cell formation during atherosclerotic lesion development is determined by a balance between the influx of proatherogenic lipoproteins and efflux of excess cholesterol to lipid-poor apo AI and HDL. The rate of macrophage reverse cholesterol transport (RCT) is a good predictor of atherosclerosis susceptibility in mice.9 Macrophage RCT is commonly measured using the model developed by Rader et al9 in which macrophage foam cells labeled with [3H]cholesterol are injected into the peritoneal cavity of mice and the appearance of the [3H]tracer in the plasma, liver, bile, and feces is determined in time. Previous studies showed that macrophage RCT was gene-dose dependently decreased in total body ABCA1 KO mice.10 This is a combined effect of inactivation of macrophage ABCA1 and the largely reduced plasma HDL levels in these animals.11 In the current study, Bi et al8 show that in vivo macrophage RCT from J774 macrophages or wild-type bone marrow–derived macrophages were not affected in LDLr KO mice lacking ABCA1 specifically in liver. At first glance, this finding seems surprising considering the large decrease in HDL-C levels in these animals. The authors suggest that the plasma HDL pool is sufficient to maintain macrophage cholesterol transport to the liver. In line, it was previously shown that pharmacological suppression of ABCA1 activity in wild-type mice by probucol treatment leading to similarly low HDL-C levels as in the HSKO mice did not negatively affect in vivo RCT from J774 macrophages.12 Probucol treatment increased the flux of [3H]-cholesterol from plasma HDL into the liver and feces, which was suggested to be the consequence of reduced ABCA1-mediated efflux of the HDL-derived cholesterol back to the plasma compartment. The detrimental effects of low HDL on RCT attributable to ABCA1 inhibition can thus possibly be overcome by induction of HDL-C delivery to the liver. However, also other factors that might influence macrophage RCT in vivo should be considered. Perhaps we are just focusing too much on HDL only. Albumin can induce efflux, although far less efficient than apo AI.13 Furthermore, the apoB lipoproteins that accumulate in the LDLr KO/HSKO mice on the atherogenic diet can function as an acceptor of the [3H]tracer from the macrophages. In line, Zanotti et al14 showed that RCT was increased when wild-type macrophages are injected into apoE KO mice despite lower HDL-C levels and normal levels of preβ-HDL. Importantly, recently it was shown that red blood cells, a major reservoir of cholesterol in blood, provide an alternative pathway for macrophage RCT.15 The relevance of this pathway is clearly illustrated by the fact that apo AI KO mice with nearly absent HDL-C levels display increased transport of effluxed cholesterol via red blood cells to the liver. It thus cannot be excluded that this pathway, combined with efflux to apoB lipoproteins and albumin, preserved the transport of the [3H]cholesterol tracer to the liver in the LDLr KO/HSKO mice.

Unfortunately, no data are published on macrophage RCT in the apoE KO/HSKO mice. HDL-C levels in the apoE KO/HSKO mice were ≈3-fold lower as compared with the LDLr KO/HSKO males, but exactly the same as in the LDLr KO/HSKO females. It is thus unlikely that the differential effects on atherosclerotic lesion development in the 2 models were the consequence of differences in HDL-C levels or HDL function. A clear difference between the 2 studies is that the effects in apoE KO mice were studied under chow conditions, whereas for the LDLr KO studies the animals were challenged with an atherogenic diet. Similarly as previously shown in the total body ABCA1 KO mice, deletion of ABCA1 specifically in the liver led to lower levels of proatherogenic apoB lipoproteins in the circulation of both apoE KO and LDLr KO mice (≈1.5-fold for both models). The absolute levels of the proatherogenic apoB lipoproteins, however, were ≈3-fold higher in the LDLr KO/HSKO mice fed the atherogenic diet as compared with the apoE KO/HSKO animals on chow. This likely explains the higher aortic cholesterol content in the LDLr KO/HSKO model and why only in this model the atheroprotective effects of hepatic ABCA1 deletion became visible.

An important factor overlooked in the current studies is the potential effect of hepatic ABCA1 deletion on blood cell counts. Atherosclerosis is a chronic inflammatory disease in response to excess lipids and blood cell counts are positively associated with atherosclerosis independent of other risk factors.16 LDLr KO mice lacking apo AI, the major apoprotein of HDL and acceptor of ABCA1-mediated efflux, display expanded populations of T, B, dendritic cells, and macrophages in lymph nodes and increased T-cell proliferation and activation.17 Furthermore, hypercholesterolemia induced by

![Figure](https://example.com/figure.png)
feeding a high-fat, high-cholesterol diet induces monocytes in experimental mouse models. Because hepatic ABCA1 KO mice, similarly as total body apo AI KO mice, virtually lack HDL, it is likely that these mice might be prone to leukocytosis, particularly when challenged with an atherogenic diet. When taking into consideration that the studies in apoE KO/HSKO mice were done while feeding regular chow diet and studies in the LDLr KO/HSKO animals after challenge with an atherogenic diet, it is conceivable that differences in inflammatory status might have influenced the differential outcome of hepatic deletion of ABCA1 on atherosclerotic lesion development in apoE KO and LDLr KO mice.

In conclusion, the study by Bi et al. not only provided an important new piece of the puzzle on the complex role of ABCA1 in lipoprotein metabolism and atherosclerosis but also raised new questions for further research. If hepatic ABCA1 can indeed be proatherogenic under specific conditions, caution is warranted with the development of novel therapeutic strategies aimed at raising HDL-C by upregulating ABCA1 expression in liver, such as anti–miR-33 therapy.

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