Editorial comment

High-density lipoprotein cholesterol levels and cholesterol efflux: a missing link?

Stefan Lorkowski[a,b] and Paul Cullen[c]

[a]Institute of Arteriosclerosis Research, [b]Institute of Biochemistry and [c]Ogham GmbH, Münster, Germany

Correspondence to Dr Stefan Lorkowski, Institut für Arterioskleroseforschung, Domagkstraße 3, 48149 Münster, Germany
Tel: +49 2 51 83 5 20 72; fax: +49 2 51 83 5 20 62;
e-mail: stefan.lorkowski@uni-muenster.de

Current Opinion in Lipidology 2004, 15:81–84

Abbreviations

ABC	ATP-binding cassette
HDL	high-density lipoprotein
LDL	low-density cholesterol
PPAR	peroxisome proliferator-activated receptor
VLDL	very low-density lipoprotein

0957-9672

Introduction

Apolipoprotein AI-mediated cholesterol efflux is a key step in reverse cholesterol transport – a concept first formulated by Glomset more than 30 years ago [1,2]. According to this model, excess cholesterol is removed from peripheral tissues by the action of apolipoprotein AI and esterified by lecithin:cholesterol acyltransferase. The resulting cholesterol esters are transported back to the liver for bile secretion, either directly in the form of high-density lipoproteins (HDLs) or after transfer to apolipoprotein B-containing lipoproteins by the action of cholesterol ester transfer protein. The model proposes that the cholesterol and lipids used for the formation and maturation of HDL derive both from non-hepatic peripheral cells and from the metabolism and remodeling of triglyceride-rich, apolipoprotein B-containing lipoproteins, and suggests that high levels of HDL cholesterol reflect a greater degree of cholesterol efflux and thus more efficient reverse cholesterol transport.

Recent results have concentrated many research groups on the study of a new candidate participant in this process – the ATP-binding cassette (ABC) transporter ABCA1. Defects in ABCA1 cause Tangier disease [3–5] – a condition characterized by reduced levels of plasma HDL cholesterol, decreased apolipoprotein AI-mediated cholesterol efflux from cells, sterol accumulation in tissue macrophages, and a possible increase in atherosclerotic risk.

The characteristics and molecular basis of Tangier disease fit with the notion that low levels of HDL cholesterol generally reflect inefficient cholesterol efflux. ABCA1 is expressed by macrophages and promotes apolipoprotein AI-mediated cholesterol efflux in healthy individuals. HDL formation occurs at the ABCA1-expressing sites in order to allow transport of excess cholesterol back to the liver and the lack of ABCA1 reduces apolipoprotein AI-mediated cholesterol efflux from peripheral cells as well as circulating levels of apolipoprotein AI and HDL cholesterol. The absence of effective apolipoprotein AI-mediated cholesterol efflux in Tangier disease also promotes cholesterol accumulation in peripheral (macrophage-rich) tissues, and, as a consequence, enhanced formation of lipid plaques. Lack of plasma HDL also reduces low-density cholesterol (LDL) in the plasma, probably because of an inability of cholesterol ester transfer protein to transfer cholesterol esters from HDL to LDL.

The classic concept of reverse cholesterol transport implies that macrophages or other non-hepatic peripheral cells contribute most, if not all, of the cholesterol in plasma HDL, so that reduced efflux from the periphery leads to low circulating HDL cholesterol. However, recent studies indicated that this may not be the case. Selective inactivation of ABCA1 in macrophages of apolipoprotein E-deficient mice markedly increased atherosclerosis and foam cell formation without altering the plasma lipids and HDL cholesterol levels, whereas complete absence of ABCA1 in apolipoprotein E or LDL receptor-deficient mice was accompanied by reduced plasma cholesterol and severe skin xanthomas characterized by marked foamy macrophages and cholesterol ester accumulation, but did not affect development, progression, or composition of atherosclerotic lesions [6]. Moreover, selective expression of ABCA1 in the macrophages of ABCA1-deficient mice resulted in only a small increase in levels of apolipoprotein AI and HDL cholesterol [7].

To study the contribution of ABCA1 on plasma lipid profiles and atherosclerosis, three independent groups recently created four different lines of ABCA1-overexpressing mice [8–10]. As nicely reviewed by Joyce *et al.* [11•], analyses of these mice do not confirm the idea that enhanced ABCA1 expression in general increases HDL cholesterol levels *in vivo*. Despite different genetic backgrounds of the mouse strains, different promoters controlling ABCA1 expression and different sources and types of the human transgene used for generating the transgenic animals, cholesterol efflux from isolated

DOI: 10.1097/01.mol.0000113215.97033.97

macrophages was increased in all three studies. However, only in the studies presented by Singaraja *et al.* and Vaisman *et al.* were plasma lipid profiles altered [9,10], being unaltered in the ABCA1-overexpressing mouse model used by Cavelier *et al.* [8].

A possible explanation for these apparently contradictory findings may be the central role of the liver in HDL formation. The liver is a major site of apolipoprotein AI and ABCA1 expression, contributing significantly to the plasma pool of nascent HDL [8,9,12–14,15•,16,17]. Moreover, selective hepatic overexpression of ABCA1 increased plasma HDL cholesterol [8,18•], while ABCA1 expressed by macrophages has only a slight effect on plasma HDL levels [6,7]. Finally, enhanced expression of ABCA1 in mouse liver by means of an adenovirus vector increased apolipoprotein AI-dependent cholesterol efflux in primary hepatocytes and raised levels of total cholesterol, phospholipids, free cholesterol, HDL cholesterol, apolipoprotein E, and apolipoprotein AI in the whole animal [19•]. Thus, one explanation for the variation in the results reviewed by Joyce *et al.* [11•] may be the relatively low expression of the human *ABCA1* transgene in the liver [11•] compared with the expression of the endogenous mouse *ABCA1* gene in the model used by Cavelier *et al.* [8]. This low level of expression of the transgene is probably responsible for the lack of change in the plasma lipid profiles.

A further interesting report investigated the retrovirus-mediated overexpression of apolipoprotein AI in apolipoprotein E-deficient bone marrow cells that were transplanted into apolipoprotein E-deficient mice with preexisting atherosclerosis [20•]. This procedure resulted in a reduction in the atherosclerotic lesions in the proximal aorta. Furthermore, apolipoprotein AI-expressing peritoneal macrophages isolated from the apolipoprotein E-deficient mice that had been reconstituted with apolipoprotein AI-overexpressing marrow showed increased expression of both ABCA1 and ABCG1. The authors speculate that the upregulation of these ABC transporters may be the main source of the antiatherogenic effect of apolipoprotein AI overexpression, although they were at a loss to explain the underlying mechanism.

Although the importance of ABCA1 expression for the regulation of the plasma HDL cholesterol level and the development of atherosclerosis has been shown in mice [8–10,21], findings in humans cast doubt on the clinical importance of ABCA1 in this respect. In a genetic study in Finns, a genetically homogeneous population with a high prevalence of coronary heart disease, for example, Kakko *et al.* [22•] found the *ABCA1* locus to be of minor importance in regulating HDL cholesterol levels. A recent study in Dutch subjects with familial hypo-α-lipoproteinemia, but without mutations in *ABCA1*, found no correlation between cholesterol efflux and apolipoprotein AI or HDL cholesterol levels [23]. A caveat must be entered in this regard, however, because other studies have shown that sequence variances in *ABCA1* may affect plasma lipids or the development or progression of atherosclerosis in certain populations [24–26].

Thus, current results indicate that ABCA1 plays a key role in hepatic cholesterol efflux, inducing pathways that modulate cholesterol homeostasis in the liver, and seem to establish the liver as a more important source of plasma HDL cholesterol than peripheral cells such as macrophages. On the other hand, increased expression of ABCA1, apolipoprotein AI, and apolipoprotein E in macrophages has been clearly shown to protect against atherosclerosis. Thus, the concept that low levels of HDL cholesterol reflect inefficient reverse cholesterol transport may be too simple. The physiological role of HDL is more complex than previously thought, and the direct effect of high HDL cholesterol levels on progression of atherosclerosis is unclear. Therefore, more specific studies and better well-defined animal models are required to understand the complexity of the reverse cholesterol transport process and to demonstrate the importance of ABCA1 as a drug target.

References

1 Glomset JA. The plasma lecithins:cholesterol acyltransferase reaction. J Lipid Res 1968; 9:155–167.

2 Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995; 36:211–228.

3 Bodzioch M, Orso E, Klucken J, *et al.* The gene encoding ATP-binding cassette transporter 1 is mutated in Tangier disease. Nat Genet 1999; 22:347–351.

4 Brooks-Wilson A, Marcil M, Clee SM, *et al.* Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. Nat Genet 1999; 22:336–345.

5 Rust S, Rosier M, Funke H, *et al.* Tangier disease is caused by mutations in the gene encoding ATP-binding cassette transporter 1. Nat Genet 1999; 22:352–355.

6 Aiello RJ, Brees D, Bourassa PA, *et al.* Increased atherosclerosis in hyperlipidemic mice with inactivation of ABCA1 in macrophages. Arterioscler Thromb Vasc Biol 2002; 22:630–637.

7 Haghpassand M, Bourassa PA, Francone OL, Aiello RJ. Monocyte/macrophage expression of ABCA1 has minimal contribution to plasma HDL levels. J Clin Invest 2001; 108:1315–1320.

8 Cavelier LB, Qiu Y, Bielicki JK, *et al.* Regulation and activity of the human ABCA1 gene in transgenic mice. J Biol Chem 2001; 276:18046–18051.

9 Singaraja RR, Bocher V, James ER, *et al.* Human ABCA1 BAC transgenic mice show increased high density lipoprotein cholesterol and ApoAI-dependent efflux stimulated by an internal promoter containing liver X receptor response elements in intron 1. J Biol Chem 2001; 276:33969–33979.

10 Vaisman BL, Lambert G, Amar M, *et al.* ABCA1 overexpression leads to hyperalphalipoproteinemia and increased biliary cholesterol excretion in transgenic mice. J Clin Invest 2001; 108:303–309.

11 • Joyce C, Freeman L, Brewer HB Jr, Santamarina-Fojo S. Study of ABCA1 function in transgenic mice. Arterioscler Thromb Vasc Biol 2003; 23:965–971.

12 Bisgaier CL, Glickman RM. Intestinal synthesis, secretion, and transport of lipoproteins. Annu Rev Physiol 1983; 45:625–636.

13 Stoffel W, Kruger E, Deutzmann R. Cell-free translation of human liver apolipoprotein AI and AII mRNA: processing of primary translation products. Hoppe Seylers Z Physiol Chem 1983; 364:227–237.

14 Langmann T, Klucken J, Reil M, *et al*. Molecular cloning of the human ATP-binding cassette transporter 1 (hABC1): evidence for sterol-dependent regulation in macrophages. Biochem Biophys Res Commun 1999; 257:29–33.

15 • Langmann T, Mauerer R, Zahn A, *et al*. Real-time reverse transcription-PCR expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. Clin Chem 2003; 49:230–238.

16 Lawn RM, Wade DP, Couse TL, Wilcox JN. Localization of human ATP-binding cassette transporter 1 (ABC1) in normal and atherosclerotic tissues. Arterioscler Thromb Vasc Biol 2001; 21:378–385.

17 Wellington CL, Walker EK, Suarez A, *et al*. ABCA1 mRNA and protein distribution patterns predict multiple different roles and levels of regulation. Lab Invest 2002; 82:273–283.

18 • Wellington CL, Brunham LR, Zhou S, *et al*. Alterations of plasma lipids in mice via adenoviral-mediated hepatic overexpression of human ABCA1. J Lipid Res 2003; 44:1470–1480.

19 • Basso F, Freeman L, Knapper CL, *et al*. Role of the hepatic ABCA1 transporter in modulating intrahepatic cholesterol and plasma HDL cholesterol concentrations. J Lipid Res 2003; 44:296–302.

20 • Su YR, Ishiguro H, Major AS, *et al*. Macrophage apolipoprotein A-I expression protects against atherosclerosis in ApoE-deficient mice and up-regulates ABC transporters. Mol Ther 2003; 8:576–583.

21 Joyce CW, Amar MJ, Lambert G, *et al*. The ATP binding cassette transporter A1 (ABCA1) modulates the development of aortic atherosclerosis in C57BL/6 and apoE-knockout mice. Proc Natl Acad Sci U S A 2002; 99:407–412.

22 • Kakko S, Kelloniemi J, von Rohr P, *et al*. ATP-binding cassette transporter A1 locus is not a major determinant of HDL-C levels in a population at high risk for coronary heart disease. Atherosclerosis 2003; 166:285–290.

23 Hovingh GK, Van Wijland MJ, Brownlie A, *et al*. The role of the ABCA1 transporter and cholesterol efflux in familial hypoalphalipoproteinemia. J Lipid Res 2003; 44:1251–1255.

24 Cenarro A, Artieda M, Castillo S, *et al*. A common variant in the ABCA1 gene is associated with a lower risk for premature coronary heart disease in familial hypercholesterolaemia. J Med Genet 2003; 40:163–168.

25 Evans D, Beil FU. The association of the R219K polymorphism in the ATP-binding cassette transporter 1 (ABCA1) gene with coronary heart disease and hyperlipidaemia. J Mol Med 2003; 81:264–270.

26 Harada T, Imai Y, Nojiri T, *et al*. A common Ile 823 Met variant of ATP-binding cassette transporter A1 gene (ABCA1) alters high density lipoprotein cholesterol level in Japanese population. Atherosclerosis 2003; 169:105–112.

Recommended reading

Aiello RJ, Brees D, Francone OL. ABCA1-deficient mice: Insights into the role of
• monocyte lipid efflux in HDL formation and inflammation. Arterioscler Thromb Vasc Biol 2003; 23:972–980.
This interesting review appeared in the *ATVB in Focus* series 'Role of ABCA1 in cellular cholesterol efflux and reverse cholesterol transport' covering topics such as reproduction difficulties, HDL, biosynthesis, foam cell accumulation and development of atherosclerosis, and cholesterol absorption in ABCA1-deficient mice.

Barter P, Kastelein J, Nunn A, Hobbs R. High density lipoproteins (HDLs) and
• atherosclerosis; the unanswered questions. Atherosclerosis 2003; 168:195–211.
The concentration of plasma HDL cholesterol has been found to be a negative predictor of premature coronary heart disease and there is experimental evidence that HDL protects against the development of atherosclerosis. However, there remain many unanswered questions that must be answered before HDL cholesterol-raising therapies are embarked on as a strategy to prevent coronary heart disease. This review highlights what is currently unknown in the story of HDL.

Basso F, Freeman L, Knapper CL, *et al*. Role of the hepatic ABCA1 transporter in
• modulating intrahepatic cholesterol and plasma HDL cholesterol concentrations. J Lipid Res 2003; 44:296–302.
This article demonstrates the importance of hepatic overexpression of ABCA1 for levels of total cholesterol, phospholipids, free cholesterol, HDL cholesterol, apolipoprotein E, and apolipoprotein AI in the plasma.

Chawla A, Lee CH, Barak Y, *et al*. PPARdelta is a very low-density lipoprotein
•• sensor in macrophages. Proc Natl Acad Sci U S A 2003; 100:1268–1273.
In this article, the authors show that very low-density lipoprotein (VLDL) functions as a transcriptional regulator in macrophages via the activation of the nuclear receptor peroxisome proliferator-activated receptor (PPAR)δ. The signaling components of native VLDL are its triglycerides, whose activities are enhanced by lipoprotein lipase. Generation of PPARδ-deficient macrophages verifies the absolute requirement of PPARδ in mediating the VLDL response. These data reveal a pathway through which dietary triglycerides and VLDL can directly regulate gene expression in atherosclerotic lesions.

Joyce C, Freeman L, Brewer HB Jr, Santamarina-Fojo S. Study of ABCA1
• function in transgenic mice. Arterioscler Thromb Vasc Biol 2003; 23:965–971.
This is a recommendable review on the existing mouse models to study ABCA1 overexpression that appeared as part of the *ATVB in Focus* series 'Role of ABCA1 in cellular cholesterol efflux and reverse cholesterol transport'. The review focuses on different aspects such as the genetic background of the models, importance of hepatic ABCA1 expression for plasma HDL levels, effect of ABCA1 overexpression on the metabolism of apolipoprotein B-containing lipoproteins and atherogenesis.

Kakko S, Kelloniemi J, von Rohr P, *et al*. ATP-binding cassette transporter A1
• locus is not a major determinant of HDL-C levels in a population at high risk for coronary heart disease. Atherosclerosis 2003; 166:285–290.
This study presents interesting findings that ABCA1 is not a major determinant for regulation of HDL cholesterol plasma levels in a homogenous Finnish population. Although, a broader study in this respect including different and larger populations is necessary to show the importance of variations in ABCA1 for risk of coronary heart disease.

Langmann T, Mauerer R, Zahn A, *et al*. Real-time reverse transcription-PCR
• expression profiling of the complete human ATP-binding cassette transporter superfamily in various tissues. Clin Chem 2003; 49:230–238.
An extensive and helpful study of the messenger RNA expression of 47 of the 49 currently known human ABC proteins in 21 different tissues using specific TaqMan reverse transcriptase polymerase chain reaction assays.

Lee CH, Chawla A, Urbiztondo N, *et al*. Transcriptional repression of atherogenic
•• inflammation: modulation by PPARdelta. Science 2003; 302:453–457.
This paper is in some respect a follow-up of the work published by Chawla *et al*. (see above). The formation of atherosclerotic lesions is mediated by lipid-laden macrophages (foam cells), which establish chronic inflammation associated with lesion progression and PPARδ promotes lipid uptake and efflux in atherogenic macrophage foam cells. The authors found that the closely related receptor PPARγ controls the inflammatory status of the macrophage. Deletion of PPARδ from mouse foam cells increased the availability of inflammatory suppressors, which reduced the area of atherosclerotic lesions by more than 50%. The authors propose a ligand-dependent transcriptional pathway in which PPARδ controls an inflammatory switch through its association and disassociation with transcriptional repressors. PPARδ ligands may therefore be interesting therapeutic targets to attenuate inflammation and to slow the progression of atherosclerosis.

Oram JF. HDL apolipoproteins and ABCA1: partners in the removal of excess
• cellular cholesterol. Arterioscler Thromb Vasc Biol 2003; 23:720–727.
This is a recent review as part of the *ATVB in Focus* series 'Role of ABCA1 in cellular cholesterol efflux and reverse cholesterol transport' covering topics such as structure and function of ABCA1, interactions between apolipoproteins and ABCA1, and regulation of ABCA1.

Su YR, Ishiguro H, Major AS, *et al*. Macrophage apolipoprotein A-I expression
• protects against atherosclerosis in ApoE-deficient mice and up-regulates ABC transporters. Mol Ther 2003; 8:576–583.
Similar to the synthesis of apolipoprotein E by macrophages, selective overexpression of apolipoprotein AI in macrophages can compensate in part for apolipoprotein E deficiency by stimulating cholesterol efflux and reverse cholesterol transport.

Wellington CL, Brunham LR, Zhou S, *et al*. Alterations of plasma lipids in mice via
• adenoviral-mediated hepatic overexpression of human ABCA1. J Lipid Res 2003; 44:1470–1480.
This study is an interesting starting point for investigating the functional roles of ABCA1 in other cell types independent of HDL cholesterol regulation.

Wickelgren I. Spinning junk into gold. Science 2003; 300:1646–1649.
•• This is a nice report about recent findings on surprising uses for the non-coding junk DNA in eukaryotic genomes: (1) introns going on to build proteins of their own despite being cut out of the pre-messenger RNA; such intron-encoded enzymes can, for example, inject new stretches of DNA into precise defined spots in a genome; (2) inteins, peptide pieces of a proteins, that are immediately and spontaneously spliced out of the proteins during protein folding; inteins can, for example, reassemble proteins or link proteins to small molecules.

Yancey PG, Bortnick AE, Kellner-Weibel G, *et al*. Importance of different
• pathways of cellular cholesterol efflux. Arterioscler Thromb Vasc Biol 2003; 23:712–719.
Beside the ABCA1-mediated cholesterol efflux pathway, two further mechanisms have been characterized by which free cholesterol can flux from cells: (1) aqueous diffusion and (2) scavenger receptor B1-mediated flux. Yancey *et al*. review nicely the three known pathways of cellular cholesterol efflux. This article was also published as part of the *ATVB in Focus* series 'Role of ABCA1 in cellular cholesterol efflux and reverse cholesterol transport'.