Editorial comment

# Genetics and molecular biology: apolipoprotein E – from plasma lipids to plaque stability

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**Abbreviations**

| | |
|---|---|
| **ABC** | ATP-binding cassette (transporter) |
| **APD** | acetyl-podocarpic dimer |
| **apoE** | apolipoprotein E |
| **CYP7A1** | cholesterol 7α-hydroxylase |
| **CYP27A1** | sterol 27-hydroxylase |
| **LXR** | liver X-receptor |
| **RXR** | retinoid X-receptor |
| **SR-BI** | scavenger receptor class B type I |
| **SREBP-1c** | sterol regulatory element-binding protein-1c |

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The identification and characterization of apolipoprotein E (apoE) by Utermann and colleagues in 1975 provided fundamental insights into lipid metabolism. In the following years, it became clear that apoE is not only a key player in lipid metabolism, but may also play a role in Alzheimer's disease, cognitive function, immunoregulation, and possibly even infectious diseases.

Mature apoE comprises 299 amino acids and is secreted mainly by the liver as a component of several classes of circulating lipoproteins. The main function of this apoE is to act as a ligand allowing the docking of triglyceride-rich lipoproteins to the apolioprotein B/apoE receptor, which is expressed on nearly all peripheral cells. However, apoE is also produced by tissue macrophages, including those located within atherosclerotic plaques. ApoE produced by plaque macrophages probably plays an important role in removing excess cholesterol from the arterial wall and thus protects against atherosclerosis.

Recently, Lafitte *et al.* [1] and Mak *et al.* [2] showed that apoE belongs to a growing group of genes, which also includes the ATP-binding cassette (ABC) transporters ABCA1 [3], ABCG1 [4–6], ABCG4 [7], ABCG5 and ABCG8 [7,8] and other proteins involved in cellular lipid metabolism such as cholesterol ester transfer protein [9], cholesterol 7α-hydroxylase (CYP7A1) [10,11], sterol regulatory element-binding protein-1c (SREBP-1c) [12], lipoprotein lipase [13], fatty acid synthase [14], and phospholipid transfer protein [15], whose expression is regulated by the nuclear receptors liver X-receptor (LXR) and retinoid X-receptor (RXR). It is of note that the expression of apoE is regulated in a similar manner to that of other proteins putatively involved in cellular cholesterol efflux such as ABCA1 and ABCG1.

Further evidence of an interaction between apoE and ABC transporters exists. Von Eckardstein *et al.* [16] showed that the putative ABCA1 inhibitor glyburide and antisense oligonucleotides directed against ABCA1 messenger RNA significantly reduced apoE secretion from human primary monocyte-derived macrophages and macrophage cells derived from the human acute monocytic leukemia THP-1 cell line. Antisense oligonucleotides directed against ABCG1 mRNA also inhibited apoE secretion, albeit to a lesser extent. In addition, apoE secretion from primary human monocyte-derived macrophages derived from ABCA1-deficient patients with Tangier disease was also decreased. ApoE mRNA expression was not affected by the inhibition of ABCA1 or ABCG1 in primary human monocyte-derived macrophages derived from healthy patients or by the lack of functional ABCA1 in primary human monocyte-derived macrophages from Tangier disease patients.

Huang *et al.* [17] compared apoE-expressing J774 macrophages ($J774E^+$) with non-expressing parental cells ($J774E^-$). Sterol efflux was higher in $J774E^+$ cells than in $J774E^-$ cells, but was not further enhanced by the cAMP-mediated induction of ABCA1 expression. The induction of ABCA1 by cAMP did, however, increase the efflux of sterol to exogenously added apolipoprotein A-I from both cell types. Inhibitors of ABCA1 activity approximately halved sterol efflux from $J774E^+$ and $J774E^-$ cells treated with cAMP and apolipoprotein A-I. In contrast to the studies of von Eckardstein *et al.* [16], however, inhibiting ABCA1 did not attenuate apoE-mediated cholesterol efflux, indicating that the increment in sterol efflux mediated by endogenous apoE in macrophages does not depend on ABCA1 expression or activity. Huang *et al.* [17] also found that increased apoE expression reduced ABCA1 expression. These results show that the relationship between ABCA1 and apoE expression in macrophages is complex and incompletely understood at present.

Mice are resistant to the development of atherosclerosis, with even the most sensitive strains such as C57BL/6 requiring a diet rich in cholesterol and the bile salt,

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sodium cholate, to develop lesions. However, it was found that obliteration of the gene for either apoE or the LDL receptor converted these animals into suitable models of the human disease. A recent and important study in this model was published in April 2002 [18]. In this work, Aiello *et al.* [18] examined whether the complete absence of ABCA1 or selected inactivation of ABCA1 in macrophages (achieved by means of bone marrow transplantation) is accompanied by the progression of atherosclerosis in hypercholesterolemic apoE-deficient or LDL receptor-deficient mice.

The absence of ABCA1 led to reduced plasma cholesterol levels in both mouse strains, and was accompanied by severe xanthomatosis, characterized by cholesterol ester accumulation in foamy macrophages of the skin. However, the complete absence of ABCA1 did not affect the development, progression, or composition of atherosclerotic lesions in ABCA1-deficient mice fed either normal chow or an atherogenic diet. By contrast, selective inactivation of ABCA1 in macrophages markedly increased atherosclerosis and foam cell accumulation in apoE-deficient mice. These findings indicate that the beneficial effect of ABCA1 obliteration on the plasma lipoprotein profile seems to counteract the proposed atherogenic effect of ABCA1 deficiency at the cellular level as a result of reduced cholesterol efflux. Selective ABCA1 deficiency in macrophages, however, is not compensated by an improved lipoprotein profile, and thus leads to increased atherosclerosis.

A central question in atherosclerosis is why some plaques rupture, thus causing heart attacks or stroke, and others remain stable for many years. Several mouse models of vulnerable atherosclerotic plaques have been reported [19–22]. However, all of these models are hampered by the long duration of the experiment, the expense required for mouse maintenance for more than one year, and the variable incidence of plaque rupture.

In a recent report [23], accelerated atherosclerosis was produced in a site-specific manner by the placement of a restrictive silastic collar around the common carotid artery of apoE-deficient mice. The resulting lesions were monocytic foam cell lesions at 3 weeks, which progressed to lesions with a necrotic core and a fibrocellular cap by 6 weeks. To induce conversion to vulnerable plaques, adenoviral vectors expressing p53 were introduced into the common carotid artery bearing collar-induced lesions. The overexpression of p53 produced a decrease in cell proliferation and an increase in fibrous cap cell apoptosis as early as one day after infection. Most striking were the reductions in cap thickness attained 14 days after infection, which were characterized by a reduction in collagen content and a loss of cap smooth muscle cells. The lesions had structural features resembling those used to define unstable plaques in human coronary arteries. Although spontaneous rupture of these p53-induced lesions only occurred in two out of 16 lesions, the incidence of plaque rupture increased to eight out of 20 lesions after intravenous injection of the vasopressor compound phenylephrine. The histological criteria for plaque rupture included intraplaque haemorrhage, cap breaks with the extrusion of core contents, intracarotid thrombosis, and phagocytosis of erythrocytes by macrophages within the lesion.

This model is an important step towards an efficient and reproducible mouse model of atherosclerosis that exhibits the salient features of spontaneous human plaque rupture. An important component of human plaque rupture that remains elusive in widely used mouse models of atherosclerosis is the formation of platelet-rich fibrin clots. Future studies are required to develop mouse models that more fully represent destabilized and ruptured advanced human atherosclerotic plaques.

It is clear that the role of apoE in intracellular lipid metabolism is at least as important as its function in the metabolism of circulating lipoproteins. Newer data show that it is part of a complex web of gene products regulating cholesterol homeostasis and efflux at the cellular level. Finally, the use of the apoE knockout mouse has fundamentally contributed to our knowledge of plaque development and rupture.

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## Recommended reading

Eichner JE, Dunn ST, Perveen G, *et al.* Apolipoprotein E polymorphism and
•• cardiovascular disease: a HuGE review. Am J Epidemiol 2002; 155:487–495.

This recent review on apoE examines the association between the apolipoprotein $\varepsilon$ gene polymorphism (or its protein product; apoE), the metabolic regulation of cholesterol and cardiovascular disease.

Ordovas JM, Mooser V. The APOE locus and the pharmacogenetics of lipid
•• response. Curr Opin Lipidol 2002; 13:113–117.

An excellent and comprehensive review of the apoE locus focusing on studies beyond traditional associations. It ties together, in particular, the findings of recent gene–environment and gene–therapy interaction studies.

Fu X, Menke JG, Chen Y, *et al.* 27-hydroxycholesterol is an endogenous ligand for
•• liver X receptor in cholesterol-loaded cells. J Biol Chem 2001; 276:38378–38387.

This study shows that sterol 27-hydroxylase (CYP27A1) is involved in the regulation of cholesterol-induced genes via the activation of LXR. The evidence for this is that (1) the introduction of CYP27A1 induced the expression of ABCG1 and SREBP-1c; (2) ABCG1 and SREBP-1c were induced more on cholesterol loading of CYP27A1-expressing cells than of control cells; (3) in CYP27A1-deficient human skin fibroblasts, the induction of ABCA1 in response to cholesterol loading was ablated; and (4) in a co-activator-association assay; 27-hydroxycholesterol functionally activated LXR.

Sparrow CP, Baffic J, Lam MH, *et al.* A potent synthetic LXR agonist is more
• effective than cholesterol loading at inducing ABCA1 mRNA and stimulating cholesterol efflux. J Biol Chem 2002; 277:10021–10027.

This work showed that the acetyl-podocarpic dimer (APD) is a selective and more potent agonist for both LXR$\alpha$ and LXR$\beta$ than endogenous oxysterols. In transient transactivation assays, APD was approximately 1000-fold more potent, and yielded approximately sixfold greater maximal stimulation than the widely used LXR agonist 22(R)-hydroxycholesterol. APD induced ABCA1 messenger RNA levels, and increased the efflux of both cholesterol and phospholipid, from multiple cell types.

Huang ZH, Mazzone T. ApoE-dependent sterol efflux from macrophages is
•• modulated by scavenger receptor class B type I expression. J Lipid Res 2002; 43:375–382.

In this work, a potential interaction between the apoE and scavenger receptor class B type I (SR-BI) sterol efflux pathways in modulating overall macrophage sterol flux was studied. In apoE-expressing cells, increased SR-BI expression reduced sterol and phospholipid efflux. An SR-BI-mediated reduction in sterol efflux was observed only in cells that expressed endogenous apoE. In J774 cells that did not express apoE a similar increase in the SR-BI level led to increased sterol efflux. The divergent response of sterol efflux after increased SR-BI was maintained in the presence of a number of structurally diverse extracellular sterol acceptors. Increased SR-BI expression also enhanced sterol efflux to exogenously added apoE. Further investigation indicated that SR-BI expression reduced macrophage apoE by accelerating the degradation of newly synthesized intracellular apoE, reducing the fraction of secreted apoE. This reduction of endogenous apoE was accompanied by reduced apoE secretion, cell surface-sequestered apoE and sterol efflux.

Bultel-Brienne S, Lestavel S, Pilon A, *et al.* Lipid free apolipoprotein E binds to
•• SR-BI and enhances cholesteryl ester uptake from lipoproteins. J Biol Chem 2002; 277:36092–36099.

In a human adrenal cell line, the authors examined the role of apoE in binding to SR-BI and in selective cholesterol ester uptake from lipoproteins to cells. This cell line does not secrete apoE, and SR-BI is its major HDL-binding protein. This work provides evidence (1) that free apoE is a ligand for SR-BI; (2) that apoE associated with lipids or in lipoproteins does not modulate the binding or selective uptake of cholesterol esters by the SR-BI pathway; and (3) that the direct interaction of free apoE with SR-BI leads to an increase in cholesterol ester uptake from lipoproteins of both low and high densities.

Akiyama TE, Sakai S, Lambert G, *et al.* Conditional disruption of the peroxisome
•• proliferator-activated receptor gamma gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux. Mol Cell Biol 2002; 22:2607–2619.

The authors produced a conditional PPAR-$\gamma$-deficient mouse using the Cre-loxP system to circumvent the embryonic lethality of PPAR-$\gamma$-deficient mice as a result of placental dysfunction. The loss of PPAR-$\gamma$ resulted in marked reductions in the basal and troglitazone-stimulated expression of genes encoding lipoprotein lipase, CD36, LXR$\alpha$, and ABCG1 in peritoneal macrophages. Reductions in the basal levels of apoE mRNA in macrophages and apoE protein in total plasma and HDL were also observed. Basal cholesterol efflux from cholesterol-loaded macrophages to HDL was significantly reduced after disruption of the PPAR-$\gamma$ gene. These data indicate that PPAR-$\gamma$ plays a critical role in the regulation of cholesterol homeostasis by controlling the expression of a network of genes that mediate cholesterol efflux from cells and its transport in plasma.

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