thematic review

Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis

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Abstract The identification of mutations in ABCA1 in patients with Tangier disease and familial HDL deficiency demonstrated that inadequate transport of phospholipid and cholesterol to the extracellular space results in the hypercatabolism of lipid-poor nascent HDL particles. However, the relationship between changes in ABCA1 activity and HDL levels is not clear. To address this question directly in vivo, we have used bacterial artificial chromosome transgenic approaches, which allow for appropriate developmental and cellular localization of human ABCA1 in mouse tissues. Increased expression of ABCA1 is directly associated with an increase in HDL levels, and the relationship between the increase in efflux and HDL is completely linear ($r^2 = 0.87$). Preliminary data have suggested that coronary artery disease (CAD) is increased in heterozygotes for ABCA1 deficiency. These results may have been biased by clinical sampling, and CAD end points are insensitive markers. We have now used surrogate end points of intima-media complex thickness (IMT) and have shown that mean IMT in ABCA1 heterozygotes is indeed increased. A strong correlation between adjusted IMT and HDL cholesterol values and apolipoprotein A-I-driven efflux has been established. These studies suggest that compromised ABCA1 activity leads to accelerated and early atherogenesis and provides a link between the cholesterol deposition in macrophages within the arterial wall and cholesterol efflux in humans.—Attie, A. D., J. P. Kastelein, and M. R. Hayden. **Pivotal role of ABCA1 in reverse cholesterol transport influencing HDL levels and susceptibility to atherosclerosis. *J. Lipid Res.* 2001.** 42: **1717–1726.**

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DEFINITION OF REVERSE CHOLESTEROL TRANSPORT

Virtually all animal cells synthesize cholesterol and import cholesterol from plasma lipoproteins and from uptake of senescent erythrocytes. To achieve neutral cholesterol balance and prevent cholesterol overload, cells must export excess cholesterol. The only quantitatively significant sink for excess cholesterol is the liver, owing to its unique ability to synthesize bile acids and to transport cholesterol into bile. The transport of cholesterol from extrahepatic tissues to the liver is termed "reverse cholesterol transport" (**Fig. 1A**).

MOLECULAR COMPONENTS

Apolipoproteins

None of the apolipoproteins is a specific cholesterol-binding protein. Rather, all are phospholipid-binding proteins (1). With the exception of apolipoprotein B (apoB), the apolipoproteins share a common amphipathic helix repeat motif that enables spontaneous binding to phospholipid (1). These interactions have been most extensively studied using apoA-I and phosphatidylcholine (PC) (2). In the absence of other lipid components, apoA-I and PC form a stable discoidal particle, termed "pre-β-HDL" (3). The increased abundance of pre-β-HDL in certain HDL deficiency states (described below) provides evidence that this particle reflects the first step in the life cycle of an HDL particle (**Fig. 2**).

Phospholipid transfer protein (PLTP)

The phospholipids on HDL particles are made available during lipolysis of triglyceride-rich lipoproteins (chylomicrons and VLDL) much of the phospholipid monolayer surface becomes redundant relative to the depleted neutral

Abbreviations: BAC, bacterial artificial chromosome; CAD, coronary artery disease; IMT, intima-media complex thickness; LXR, liver X receptor; TD, Tangier disease.

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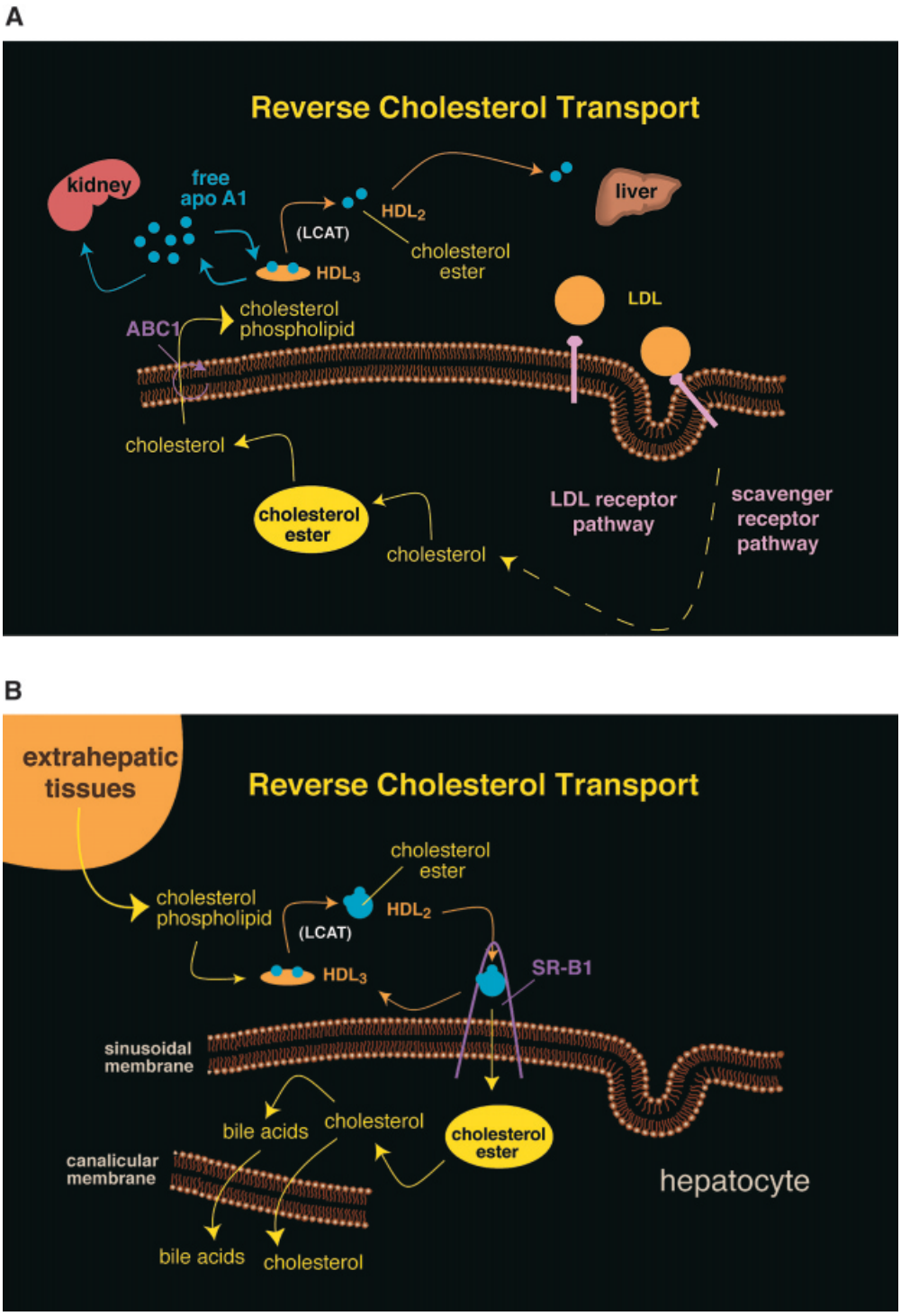

Fig. 1. A: Reverse cholesterol transport. Extrahepatic tissues synthesize cholesterol and also derive cholesterol through the uptake of lipoproteins via the LDL receptor and scavenger receptors. The cholesteryl ester is in a dynamic equilibrium with free cholesterol, through the opposing actions of ACAT and neutral cholesterol esterase. Free cholesterol effluxes to extracellular acceptors, most notably phospholipid/apoA-I disks (pre-β-HDL). This process is directly (or indirectly through phospholipid efflux) dependent on functional ABCA1. Proper lipidation is essential for the stability of HDL. In the absence of sufficient cholesterol efflux, apoA-I is rapidly cleared from the circulation by the kidneys. Cholesterol that associates with apoA-I/phospholipid disks is a substrate for LCAT. LCAT transfers a fatty acyl chain from phosphatidylcholine to cholesterol, forming cholesteryl ester. The cholesteryl ester partitions into the hydrophobic core of the lipoprotein, thus forming spherical HDL particles. These particles can then deliver cholesteryl ester to the liver and steroidogenic tissues. B: Selective uptake of cholesteryl esters from HDL. The interaction of spherical HDL particles with the scavenger receptor class B type I (SR-BI) leads to selective delivery of cholesteryl esters. SR-BI interacts with spherical HDL particles but not with apoA-I or poorly lipidated HDL disks. The cholesteryl esters are hydrolyzed by a neutral cholesterol esterase, providing free cholesterol for secretion across the apical (bile canalicular) membrane of the hepatocyte and for bile acid synthesis. Although the diagram shows cholesterol coming from extrahepatic tissues, growing evidence suggests that a major source of cholesterol for ABCA1-mediated transport to HDL is the liver.

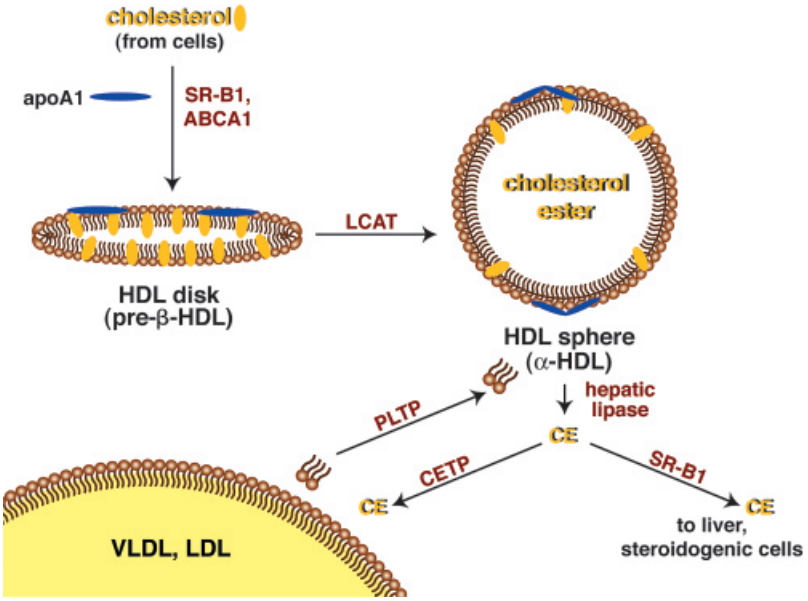

Fig. 2. Factors affecting the size and stability of HDL particles. ApoA-I spontaneously binds to phospholipids to form disklike particles. ABCA1 is necessary for phospholipid efflux to apoA-I. The capacity of cells to efflux cholesterol to acceptors (e.g., apoA-I/phospholipid disks) is correlated with their content of SR-BI. Surface phospholipid is derived from the surface of triglyceride-rich lipoproteins through the action of phospholipid transfer protein (PLTP). The gain or loss of cholesteryl esters from these particles determines their residence time in the circulation. Cholesteryl esters are derived from the LCAT reaction—LCAT promotes the conversion of HDL disks (pre-β-HDL) to spherical HDL. Cholesteryl esters are removed from HDL particles by two processes: *1*) the transfer of cholesteryl ester to VLDL and LDL, catalyzed by CETP, and *2*) the selective uptake of cholesteryl esters into hepatocytes and steroidogenic cells via the SR-BI. The hydrolysis of surface phospholipids by hepatic lipase enhances the loss of cholesteryl esters by both CETP and the SR-BI.

lipid core (4). This surface becomes available to form HDL precursor particles (Fig. 2). PLTP facilitates the formation of pre-β-HDL particles from this phospholipid source (5). Loss of PLTP results in 60–70% HDL deficiency in mice (6). The sera from these mice are essentially unable to catalyze transfer of phospholipids from VLDL to HDL (6). The reduction in HDL caused by PLTP deletion is comparable to that caused by the loss of VLDL and chylomicrons in abetalipoproteinemia, a disease caused by mutations in microsomal triglyceride transfer protein (7). Thus, it is likely that the phospholipid substrate of PLTP is primarily in VLDL and chylomicron particles. However, that accounts for only about half of the phospholipid used to produce HDL particles.

LCAT

Although apolipoproteins cannot bind to cholesterol, apolipoprotein/phospholipid particles are cholesterol sinks when they are in the presence of a cholesterol-rich particle or membrane. The pioneering studies of Glomset (8) showed that the plasma enzyme LCAT catalyzes the transfer of an acyl chain from PC to cholesterol, forming cholesteryl ester (Figs. 1A and 2). Cholesteryl ester, unlike cholesterol, is not amphipathic and therefore partitions into the core of a discoidal particle (8). This enables the particle to accept a substantial amount of cholesterol from virtually any cholesterol donor. The cholesteryl ester-enriched HDL particle that results from LCAT action is termed "α-HDL." Deletion of LCAT in transgenic mice results in >90% loss of HDL, owing to the inability to create mature, stable particles (9). In humans, LCAT deficiency also results in HDL deficiency. In some patients, there is also corneal opacity (fish eye disease), renal disease, and anemia [reviewed in ref. (10)].

CETP

CETP catalyzes the reciprocal exchange of cholesteryl ester for triglyceride between HDL and VLDL (5). CETP contributes to reverse cholesterol transport in a pathway that involves cholesteryl ester transport from HDL to VLDL and then to the liver through VLDL remnant clearance (Fig. 2). However, kinetic studies (11) as well as the virtual absence of CETP in mice and rats (12) suggest that this pathway probably does not contribute to cholesterol flux to the liver as much as does the scavenger receptor class B type I (SR-BI) pathway. CETP deficiency is associated with high HDL levels in humans (13) and with reduced HDL levels in transgenic mice expressing human CETP (14). This occurs because the most important contributor to HDL stability is its cholesteryl ester content; cholesteryl ester-rich HDL is quite stable (15).

CETP activity is responsible for the well-known inverse relationship between plasma triglyceride levels and HDL cholesterol in hypertriglyceridemic individuals. Individuals with hypertriglyceridemia (e.g., diabetics) typically have low HDL levels (16). CETP catalyzes the exchange of VLDL triglyceride for HDL cholesteryl ester, resulting in HDL particles with a triglyceride core that can then be a substrate for lipoprotein lipase and hepatic lipase (17–19). The latter enzymes then reduce the size of the particles and render them unstable. Interestingly, diabetic rats, although hypertriglyceridemic, do not have low HDL, a consequence of their deficiency in CETP (20).

SR-BI

The work of Pittman broke ground in the development of our current understanding of cholesterol delivery to the liver. Pittman's laboratory (21) showed that the uptake of cholesteryl ester in HDL by hepatocytes and steroidogenic cells was much higher than that of apoA-I, a process termed "selective uptake" of cholesterol. Confirmation of Pittman's model came from the discovery of SR-BI and the work showing that this receptor binds to HDL and mediates selective uptake of cholesteryl ester from HDL to hepatocytes and steroidogenic cells (22–24) (Fig. 2). Early work by Schwartz and co-workers (25) suggested that HDL cholesterol is targeted to the bile to a greater extent

than LDL cholesterol. In agreement with this work, mice overexpressing SR-BI have increased transport of HDL cholesterol to the bile (23).

Through mechanisms still poorly understood, SR-BI promotes cholesterol efflux from cells when there is a favorable cholesterol gradient (26) (Fig. 2). There is some controversy surrounding the question of whether HDL must physically bind to SR-BI in order to mediate cholesterol efflux. Rothblat and co-workers (27) compared HDL binding to SR-BI and to another scavenger receptor, CD36. They showed that although HDL binding to CD36 is greater than its binding to SR-BI, only the latter promoted significant cholesterol efflux. Moreover, there was more cholesterol efflux to phospholipid vesicles (which do not bind to surface receptors) in SR-BI-expressing cells than in CD36-expressing cells (27). These results led to the conclusion that SR-BI promotes cholesterol efflux through a process not dependent on the tethering of HDL to its receptor (27). Instead, Rothblat and co-workers have suggested that SR-BI elicits a redistribution of cellular free cholesterol to a pool that is more accessible to cholesterol oxidase (and presumably also to an extracellular cholesterol acceptor) (28). An alternative model has been advanced by Krieger and co-workers (29). They showed that a mutant form of SR-BI that abolishes binding to HDL, but not LDL, also abrogates cholesterol efflux to HDL and not LDL. On the basis of these observations, Krieger and coworkers assert that HDL binding to SR-BI is required for cholesterol efflux.

Hepatic lipase

Hepatic lipase is a cell surface-bound (and in the mouse, secreted) enzyme capable of hydrolyzing phospholipids and triglycerides in plasma lipoproteins (30). Because there is much more phospholipid than triglyceride in HDL, the phospholipase activity of hepatic lipase is likely the most physiologically relevant action of this enzyme in HDL metabolism. Through its depletion of HDL phospholipid, hepatic lipase facilitates the removal of cholesteryl ester from HDL through CETP (31) and through selective cholesteryl ester uptake into cells (32, 33). There is also evidence for a nonenzymatic role of hepatic lipase in promoting cellular uptake of HDL cholesterol; an enzymatically inactive form of the enzyme still promotes selective uptake of HDL cholesteryl ester in vivo (34).

ABCA1

A large body of work has identified an intracellular pathway by which apoB-containing lipoproteins, chylomicrons and VLDL, are assembled within the secretory pathway and secreted as mature lipoprotein particles (35). The earliest indication that protein and lipid are brought together within the secretory pathway was the detection by electron microscopy of lipoprotein particles within the secretory pathway (36). Yet, there was no evidence for intracellular assembly of HDL, opening the possibility that the raw materials necessary for HDL assembly are separately secreted and that HDL assembly is de facto an extracellular event. Evidence that the export of HDL lipid and apoA-I is not necessarily coupled came from studies of the WHAM chicken, an animal model of ABCA1 dysfunction (37). Isolated hepatocyte experiments established that apoA-I secretion in the WHAM chicken occurs at the same rate as in control animals (38). Yet, there is a 95% reduction in HDL levels in the WHAM chicken. Turnover studies showed that when ^{125}I-labeled HDL was injected into the WHAM chickens, its turnover was increased, but not enough to account for the HDL deficiency of these animals. However, when lipid-free ^{125}I-labeled apoA-I was injected, it disappeared at a rate that does account for the dearth of HDL in the WHAM chicken (38). This, together with a phospholipid deficit in WHAM plasma, suggested that an extracellular lipidation step is rate-limiting for HDL stability and is defective in the WHAM chickens.

Cholesterol and phospholipid efflux experiments using fibroblasts from patients with Tangier disease (TD) provided much more detailed information about this lipidation process. With HDL as a cholesterol acceptor, there is little difference in cholesterol efflux between normal and Tangier fibroblasts (39), but a large difference in phospholipid efflux. However, with lipid-free apoA-I as the acceptor, there is a pronounced reduction in cholesterol efflux from the Tangier cells (39, 40). The amount of cholesterol that effluxes from cells when HDL is the acceptor is far greater than the amount that effluxes when apoA-I is the acceptor (39). These results support the following working model for cholesterol efflux: *1*) There exists a high specificity, low capacity lipid efflux pathway that is ABCA1 dependent together with a high capacity, low specificity, ABCA1-independent lipid efflux pathway; *2*) There is no evidence that cholesterol is the primary substrate of ABCA1. There is much indirect evidence that phospholipid is a primary substrate and that cholesterol is transported through a spontaneous diffusion-mediated process after sufficient phospholipid has been complexed with apolipoprotein to create a good cholesterol sink [e.g., see ref. (41)]; and *3*) the HDL deficiency that occurs in individuals with ABCA1 dysfunction is a consequence of inadequate transport of phospholipid to the extracellular space and the resulting hypercatabolism of lipid-poor apoA-I (Fig. 1B). The cholesterol storage disorder that occurs with ABCA1 mutations might possibly be more a consequence of HDL deficiency than a direct consequence of dysfunctional ABCA1.

The ABCA1 mutations allow us to ask, what are the tissue sources of lipids that are transported to HDL in an ABCA1-dependent fashion? There is a paucity of autopsy data from humans with TD. However, tissues from several of these patients have been analyzed (42–44). The most dramatic cholesteryl ester accumulation was seen in macrophages in the spleen, the lamina propria of the intestine, and in Kupffer cells. In one study, there was also cholesteryl ester accumulation in hepatocytes (44). In the WHAM chicken, there is dramatic cholesteryl ester accumulation in hepatocytes, and tissue macrophages in the spleen and the intestine (A. D. Attie and M. R. Hayden,

unpublished observations). ABCA1 knockout mouse models have been developed in two different strain backgrounds, DBA/1J and C57BL/6J (45–47). In both, there is pronounced macrophage accumulation of cholesteryl ester in the lung. However, in the DBA/1J background, there was little evidence of a lipid storage disease in other tissues (45). In the C57BL/6 strain, there was lipid accumulation in the liver, thymus, and testes (47). Liver perfusion experiments (48) as well as mesenteric lymph collection (49) showed that the liver and the intestine release lipidated HDL particles. These results, together with the cholesteryl ester accumulation in the livers and intestines of Tangier patients (44) and WHAM chickens (A. D. Attie and M. R. Hayden, unpublished observations) suggest that ABCA1 in one or both of these tissues may contribute significantly to HDL lipids. Alternatively, tissue macrophages might also make a significant contribution to HDL lipids. This latter concept has been experimentally tested. Bone marrow transplants were carried out between wild-type and ABCA1-null mice, with each type of mouse serving as either donor or recipient on a 2×2 factorial design. After 3 months, none of the animals showed a substantial change in HDL levels, suggesting that macrophages do not contribute significantly to ABCA1-mediated lipid transport to HDL (R. J. Aiello, personal communication). This leaves the liver and the intestine as the most likely candidates.

Lipid-poor apoA-I is the preferred acceptor of ABCA1-mediated phospholipid/cholesterol efflux (40, 50) (Fig. 1B). Although controversial (51), it has been suggested, on the basis of cross-linking experiments, that apoA-I specifically binds to ABCA1 (50, 52). Mature HDL, on the other hand, preferentially interacts with SR-BI, and in so doing delivers much of its cholesteryl ester load (23, 24). Thus, it appears that a circular pathway exists in the liver whereby cholesterol is transported to apoA-I/phospholipid particles (pre-β-HDL) in an ABCA1-dependent fashion. The cholesterol is esterified by LCAT and the particles mature to become spherical α-HDL. This particle can then bind to SR-BI and unload its cholesteryl esters, thus regenerating an acceptor for ABCA1 pathway-derived cholesterol, a process that has been modeled in cultured 293 cells (53). Studies suggest that SR-BI can specifically target bound HDL protein and lipid from the sinusoidal to the canalicular surface, thus transporting cholesterol to the bile (54). Within this pathway, a neutral cholesterol esterase is thought to hydrolyze the cholesteryl esters. These findings can be placed into a model in which cholesterol transport to bile involves ABCA1-mediated efflux followed by SR-BI-mediated reuptake.

RELATIONSHIP BETWEEN INCREASED ABC1 EXPRESSION AND HDL LEVELS

It is important to make a distinction between lipid efflux processes that are rate limiting for HDL stability and those processes that are quantitatively important in cholesterol efflux. The collective evidence from turnover studies in humans (55) and transgenic mouse experiments (9, 56–59) supports the generalization that factors increasing HDL size lead to increased steady state HDL levels whereas those that reduce HDL size lead to reduced HDL levels, primarily because of increased HDL turnover. For example, two processes that deplete the cholesteryl ester core of HDL are CETP-mediated cholesteryl ester transfer to VLDL and SR-BI-mediated selective uptake of cholesteryl ester into cells. It follows that overexpression of either CETP or SR-BI leads to a reduction in HDL size and to an increase in its catabolism. Conversely, deficiency of CETP or SR-BI produces larger, more stable HDL particles. As described below, these two deficiencies appear to have opposite effects on atherosclerosis even though they have similar effects on HDL levels.

In patients with HDL deficiency due to mutations in the *ABCA1* gene, there is a highly significant correlation between cholesterol efflux and HDL levels, with efflux levels accounting for 82% of the variation in HDL in these families (60). Using regression analysis, an estimation of the relationship between ABCA1 efflux activity and HDL levels was made, which predicted that each 8% change in efflux would be associated with a 0.1 mM change in HDL cholesterol levels. These data suggest that increasing efflux would be associated with changes in HDL levels but thus does not directly address the question as to the direct relationship between efflux and HDL levels in vivo.

Three different groups have tried to directly address this question in vivo. Two groups (61, 62) have chosen to use bacterial artificial chromosome (BAC) transgenic approaches that utilize the endogenous regulatory elements, which should account for appropriate developmental, cellular, and subcellular localization of ABCA1 in mouse tissues. Interestingly, both groups showed increases in apoA-I-mediated cholesterol efflux in response to an increase in cellular cholesterol content. However, the changes in efflux were not accompanied directly by alterations in plasma HDL in the study by Cavelier et al. (62). These data were surprising and unexpected, by virtue of the previously demonstrated strong correlation between efflux and HDL levels in humans (63). Numerous explanations have been proposed to account for this finding, including the fact that there may be a minimal rate of efflux that is necessary to affect the overall flux of HDL and that the increased efflux in these animals was not beyond the critical level to affect HDL. Alternatively, this also suggests that plasma levels of HDL may be only one, limited measure of reverse cholesterol transport in cells. For example, increased flux of HDL associated with increased reverse cholesterol transport may not be associated with an increase in HDL if at the same time uptake by SR-BI in the liver is increased. Furthermore, the mice described in these two articles were created on different backgrounds. Strain differences in HDL and lipid metabolism in response to a high fat diet are well documented. Strain differences could contribute to alterations in HDL by having different levels of apoA-I or by other factors not yet understood that affect the turnover of various HDL species.

The article by Singaraja et al. (61) described BAC trans-

genic mice including intron 1, but without the endogenous promoter. The significant increase in HDL levels in these BAC transgenic mice fed both chow and atherogenic diets indicates that the alternate promoter in intron 1 is sufficiently functional to result in increased expression of ABCA1 protein and increased HDL levels. Interestingly, this was associated with increased efflux not only in macrophages, but also in fibroblasts. The ability to respond to a high fat diet in these animals was shown to be, in all likelihood, responsive to liver X receptor (LXR) elements in intron 1. The relationship between the increase in efflux and increase in HDL appeared to be completely linear, with a correlation coefficient (r^2) of 0.87 ($P = 0.007$) showing that raised efflux levels are now conclusively directly associated with a proportionate increase in HDL in vivo. The increase in efflux was almost completely mediated by the increase in ABCA1 protein, with an almost perfect correlation between efflux and ABCA1 protein levels in these animals ($r^2 = 0.98$). These data provide direct proof that any increase in net functional ABCA1 protein would be expected to have a proportionate increase in cholesterol efflux and ABCA1 levels.

Vaisman et al. (64) have also generated ABCA1 transgenic mice, using a cDNA approach under the control of the human apoE promoter, which would direct expression to hepatocytes and to the macrophage. These investigators also showed that there was a significant delay in HDL turnover due to enhanced ABCA1-mediated cellular cholesterol efflux. This was also associated with an increase in cholesterol and phospholipid content of bile.

Evidence for the protective effect of increased ABCA1 activity and changes in lipid levels and modified risk for coronary artery disease (CAD) in humans is not yet available. However, there are numerous pieces of indirect evidence, which support that the findings in mice can be extended to humans. We have assessed whether variants in the *ABCA1* gene in humans, which are frequent in the general population, can influence plasma lipid levels and risk for CAD in the adult human population. We have identified one such single nucleotide polymorphism, the SNP R291K variant, which has a carrier frequency of 46% in Europeans and is associated with a reduced severity of CAD manifested by less evidence for CAD by angiographic measurements, and also associated with fewer clinical events in carriers of this particular SNP. Furthermore, and interestingly, atherosclerosis progresses more slowly in carriers of the R291K than in noncarriers (63). Carriers have alteration in lipid levels with a decreased triglyceride levels and a trend toward an increased HDL (63). These findings are most consistent with a gain of function effect of this variant on ABCA1 activity. Preliminary evidence did not reveal any obvious difference in cholesterol efflux levels. This result was probably influenced by the low numbers assessed and high interassay coefficient of variation in the efflux assay, which would make it impossible to detect small differences in efflux. Nevertheless, these findings would suggest that, similar to mice, increased ABCA1 activity may be associated with significant alteration in lipoprotein values, and predict a decrease in CAD.

RELATIONSHIP TO ATHEROSCLEROSIS

There is a wide consensus that cholesterol and/or cholesteryl ester accumulation in macrophages plays a role in atherogenesis and that this process occurs through an inflammatory process (65). A corollary to this premise is that factors that affect the balance between cholesterol retention and cholesterol efflux in macrophages will be pro- or antiatherogenic.

Epidemiological studies establish a strong negative correlation between HDL levels and risk of premature atherosclerosis (66). Glomset (8) first proposed that the primary antiatherogenic function of HDL might be related to its key role in the transport of cholesterol from peripheral cells to the liver. However, until recently, little has been understood about the initial step of reverse cholesterol transport, namely, the removal of cholesterol from peripheral cells. Yet, if HDL is antiatherogenic because it mediates removal of cholesterol from macrophages and other cells relevant to atherogenesis, then one might predict that only those factors that increase HDL levels due to enhanced lipid transfer to HDL are antiatherogenic. Conversely, factors that increase HDL levels due to a bottleneck in HDL cholesteryl ester clearance by the liver might be atherogenic (67). These considerations predict that reverse cholesterol transport is not necessarily correlated with HDL levels. This prediction is borne out in several ways: *1*) indirect measurements of in vivo cholesterol efflux (measured in terms of compensatory increases in cholesterol synthesis) show no relationship to HDL levels in several transgenic mouse models (68); *2*) in SR-BI-deficient mice, HDL levels are elevated yet the animals have increased atherosclerosis (69). The converse occurs in SR-BI-overexpressing mice (70, 71). In the latter example, an HDL deficit as a consequence of increased selective uptake to the liver reflects increased reverse cholesterol transport; and 3) CETP deficiency in humans has in some cases been associated with increased HDL and increased risk of coronary heart disease (72), again consistent with a dissociation between a marker of reverse cholesterol transport and steady state HDL levels.

With ABCA1 deficiency, apoA-I is rapidly cleared before it is able to acquire cholesterol (38, 73). Thus, unlike the hypercatabolism of HDL that occurs with SR-BI overexpression, the loss of HDL in ABCA1 deficiency is not accompanied by the benefit of enhanced cholesterol clearance. This might account for the severe cholesteryl ester storage phenotype seen in tissue macrophages and in hepatocytes of Tangier patients and WHAM chickens.

In contrast to ABCA1 deficiency, PLTP deficiency leads to reduced HDL without a cholesteryl ester storage disorder (6). In fact, PLTP knockout mice are protected from atherosclerosis, perhaps because of a reduction in secretion of apoB-containing lipoproteins (74).

CAD IN ABCA1 HETEROZYGOTES

As many factors, both genetic and environmental, influence plasma HDL cholesterol levels and contribute to low

HDL cholesterol values, unambiguous identification of heterozygotes for *ABCA1* mutations has until now been impossible. Individuals from TD kindreds presumed to be heterozygous have shown a range of phenotypes, and much overlap with unaffected individuals has been seen (75–77), possibly reflecting the fact that some individuals had been misclassified. Indeed, the inability to uniquely identify heterozygous individuals created difficulty in mapping the gene for TD. Studies in obligate heterozygotes have also been limited to small numbers (78), often within a single family (75), and thus restricted in the ability to analyze the phenotypic expression with different mutations and over a range of ages.

We have identified a large cohort of individuals in whom heterozygosity has been defined by mutation identification in the *ABCA1* gene (60). For the first time, it became possible to characterize the phenotype in mutation-defined heterozygotes and to compare this with a large number of unaffected family members, thus controlling for other genetic and environmental factors. Our initial cohort comprised 77 individuals from 11 families identified as heterozygotes for mutations in the *ABCA1* gene. A comparison of mean lipid levels in heterozygotes with mean levels in all available unaffected family members (n = 156) is presented in **Table 1**. Heterozygotes have an approximately 40–45% decrease in HDL-C and apoA-I and a mild decrease in apoA-II compared with unaffected family members. Mean TG were increased by approximately 40% in heterozygotes compared with unaffected family members. Mean HDL cholesterol levels in *ABCA1* mutation carriers were similarly reduced by approximately 40–50% compared with unaffected family members.

Another important question is whether individuals heterozygous for *ABCA1* mutations are at an increased risk of developing CAD. Studies of obligate TD heterozygotes have reported conflicting findings (75, 78). In our large cohort, symptomatic vascular disease was more than three times as frequent in the adult heterozygotes as in unaffected family members. Interestingly, the presentation of vascular disease was generally more severe in the heterozygotes than in their unaffected family members and the mean age of onset was on average a decade earlier in heterozygotes compared with unaffected control subjects.

We next assessed the relationship between cholesterol efflux levels, HDL cholesterol, and CAD. We have previously shown that individuals heterozygous for *ABCA1* mutations have decreased cholesterol efflux (79); however, the extent to which variations in cholesterol efflux are directly related to HDL cholesterol levels was unknown. Relative cholesterol efflux in individuals heterozygous for an *ABCA1* mutation was correlated with the mean HDL cholesterol levels observed in the carriers of that mutation. Using the regression equation of mean HDL cholesterol levels in the heterozygotes on the efflux level of the heterozygous carrier, the relationship between expected changes in ABCA1 efflux activity and HDL cholesterol levels could be calculated. From this, we would predict that each 8% change in efflux levels would be associated with a 0.1 mM change in HDL cholesterol. Relative cholesterol efflux levels are also related to CAD within the family. Families with clearest evidence for premature CAD had individuals with the lowest cholesterol efflux. These data suggest that the level of residual ABCA1 function is a critical determinant of both HDL cholesterol levels and risk of CAD.

But because the collection of these kindreds may have been biased by clinical sampling and because CAD end points are insensitive markers for atherosclerosis, we used the surrogate end point of intima-media complex thickness (IMT) of peripheral arteries in these patients. In this way direct relationships between ABCA1 function and atherosclerotic wall abnormalities could be elucidated (80).

Mean IMT in ABCA1 heterozygotes was indeed increased compared with control subjects, and increased at a greater rate per year in persons with *ABCA1* mutations. From these estimates, it was calculated that heterozygotes for the *ABCA1* mutation reach an IMT of 0.75 mm at age 55 years compared with age 80 years in unaffected control subjects, indicating a much more rapid initiation and progression of atherogenesis (80). Because IMT is a validated surrogate marker for atherosclerosis, this extends our findings of earlier onset of CAD in ABCA1 heterozygotes. **Figure 3B** shows an example of the abnormal arterial wall (carotid artery) of an ABCA1 heterozygote (Fig. 3A shows the normal arterial wall of his unaffected sibling).

Our findings demonstrated that ABCA1 heterozygotes possess significantly larger mean IMT than control sub-

TABLE 1. Characterization of ABCA1 heterozygotes and unaffected siblings

	Heterozygotes	Unaffected Family Members	*P*
Number	77	156	
Age (years)	42.5 ± 19.6	39.9 ± 21.0	NS
TC (mM)	4.52 ± 1.12	4.71 ± 1.07	0.23
TG (mM)	1.66 ± 1.59	1.20 ± 1.03	0.03
HDL (mM)	0.74 ± 0.24	1.31 ± 0.35	<0.0001
LDL (mM)	3.03 ± 0.99	2.84 ± 0.87	0.171
ApoA-I (g/l)	0.92 ± 0.32 (61)	1.43 ± 0.26 (55)	<0.0001
ApoA-II (g/l)	0.35 ± 0.08 (46)	0.39 ± 0.08 (43)	0.01
ApoB (g/l)	0.93 ± 0.25 (52)	0.94 ± 0.33 (42)	0.88
CHD ≥20 years	12.9% (8/62)	4.1% (5/122)	0.03
Odds ratio (95% CI)			3.47 (1.08–11.09)
Age of onset	48.9 ± 8.6	60.4 ± 12.8	0.08

Abbreviations: TC, Total cholesterol; NS, not significant; CHD, coronary heart disease.

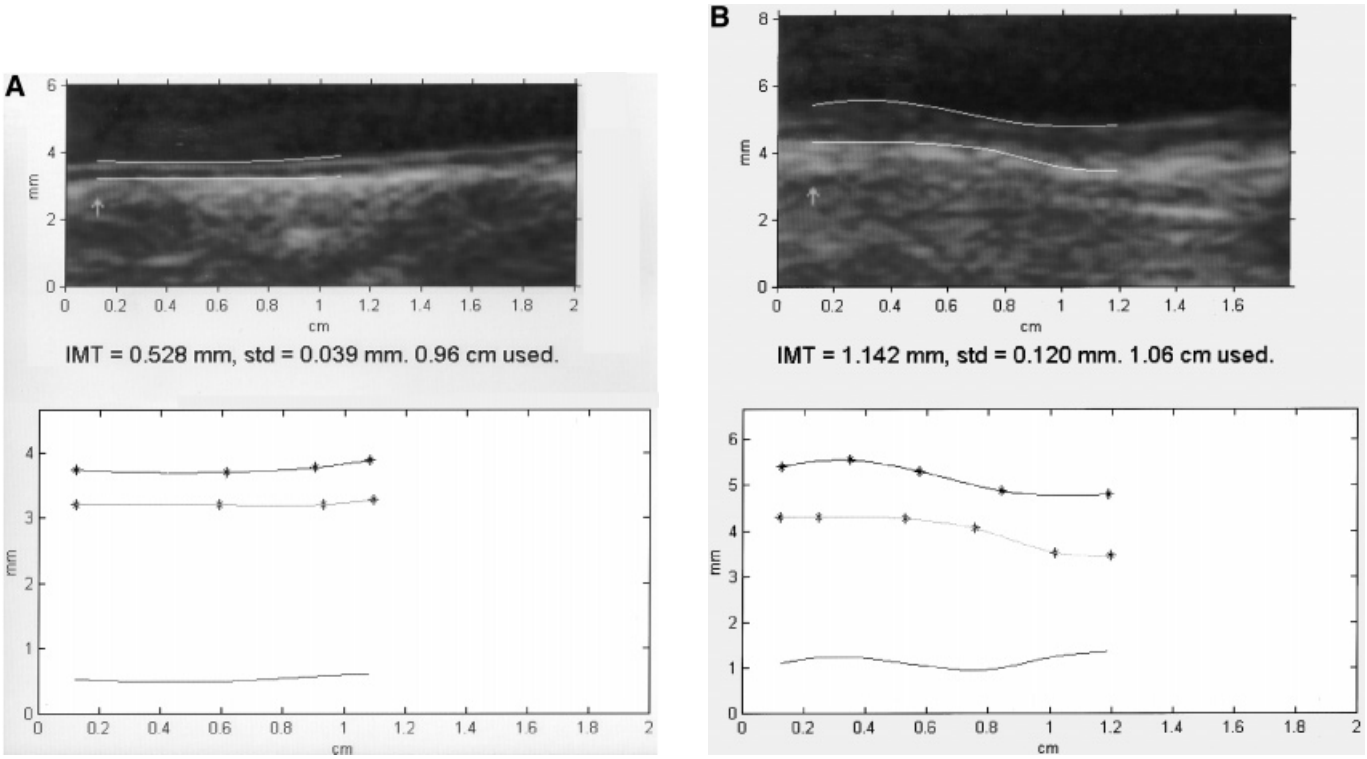

Fig. 3. A: IMT measure of the distal common carotid of an unaffected male sibling (aged 40 years) of a heterozygote for a mutation in the *ABCA1* gene, showing no obvious obstruction or thickening of the intima. B: IMT measure of the distal common carotid of a 42-year-old male with a mutation in the *ABCA1* gene, showing extensive thickening and plaque formation within the intima on both walls of the artery. This is a sibling of the brother represented in (A).

jects and therefore have increased arterial wall thickness (Fig. 3A and B). Even more striking, a strong correlation was observed between levels of cholesterol efflux in skin fibroblasts and mean arterial wall IMT. These findings show that compromised ABCA1 activity leads to accelerated and early atherogenesis and suggest that HDL-mediated removal of excess cholesterol from the arterial wall would be associated with atheroprotection.

Our data therefore provide a link for the sequence of events starting with cholesterol deposition in macrophages in the arterial wall, followed by ineffective cholesterol efflux and lack of protection against the LDL-driven increase in the intima of the arterial wall. Our findings suggest that therapies designed to promote cholesterol efflux, raise HDL, and increase reverse cholesterol transport may have a significant therapeutic benefit. It can be predicted from the steep correlations between efflux, HDL levels, and IMT that small changes in efflux will have a measurable impact on arterial wall thickness and therefore on the prevalence of CAD.

Clearly, mutations in *ABCA1* predispose and are associated with accelerated premature atherosclerosis. This is also associated with reduced HDL levels and decreased cholesterol efflux from peripheral cells. Whether this enhanced atherosclerosis is a direct result of a low HDL level or a consequence of dysfunction of ABCA1 in macrophages independent of HDL levels is not yet clear.

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