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Genetic control of apoprotein A-I and atheroprotection: Some insights from inbred strains of mice

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Abstract

Purpose of review—Previous epidemiological studies and studies in experimental animals have provided strong evidence for the atheroprotective effect of HDL and its major apoprotein, apoA-I. Identification of genetic loci associating apoA-I/HDL with cardiovascular disease is needed to establish a causal relationship.

Recent findings—Pharmacological interventions to increase apoA-I or HDL cholesterol levels in humans are not associated with reduction in atherosclerosis. GWAS studies in humans and hybrid mouse diversity panel (HMDP) studies looking for genetic variants associated with apoA-I or HDL-cholesterol levels with cardiovascular disease and atherosclerosis have not provided strong evidence for their atheroprotective function.

Summary—These findings indicate that GWAS and HMDP studies identifying possible genetic determinants of HDL and apoA-I function are needed.

Keywords

HDL; apoA-I; atherosclerosis; cardiovascular disease

INTRODUCTION

In human epidemiological studies, there is a well-established inverse relationship between plasma HDL levels, usually measured as HDL cholesterol (HDL-C), and cardiovascular disease (CVD) that is observed at all levels of plasma LDL. However, attempts to manipulate HDL levels by various therapeutic interventions have yielded mixed clinical outcomes and this has cast doubt on whether HDL-C level is the best indication of the pathophysiological importance of this lipoprotein. As a result, attention has turned instead to the assessment of HDL function. There are many potential functions of HDL that could contribute to its capacity to protect against the development of atherosclerotic heart disease, including anti-inflammatory, anti-oxidative, anti-apoptotic and anti-thrombotic functions, the

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Conflicts of interest

None.

promotion of nitric oxide production in the artery wall, and its best-characterized function the capacity to promote cholesterol efflux from the lipid-loaded cells of the plaque [1]*. Indeed, HDL from patients with CVD is less effective in promoting cholesterol efflux *in vitro*. These various functions are not mutually exclusive.

HDL is not a homogeneous lipoprotein, especially in humans. Not only do subclasses of HDL with different sizes co-exist in the plasma, but analysis of the proteins on HDL shows that the lipoprotein contains many proteins, exhibiting different functional attributes associated with cholesterol homeostasis, immune function, proteolysis, complement and anti-oxidation [2, 3]. Many of these proteins are present in sub-stoichiometric amounts, indicating that these proteins do not all necessarily inhabit the same particle. Presumably the precise protein composition of the subspecies of HDL particles influences their function. Not only is there heterogeneity of the protein composition among subspecies of HDL, but the lipid composition is also heterogeneous [4]. The complexity of HDL structure and function may be indicative of why a causal link of HDL to CVD has been difficult to identify.

This complexity notwithstanding, one protein is common to most HDL particles; namely apolipoprotein A-I (apoA-I). It comprises >70% of total HDL protein and there are 2–4 apoA-I molecules per HDL particle. Besides serving as a structural component of HDL, apoA-I, through interaction with the cell surface protein ABCA1, is essential for the biogenesis of HDL and has a major role in the promotion of cholesterol and phospholipid efflux from lipid loaded cells, particularly macrophages. The most robust evidence for the atheroprotective function of HDL derives from the overexpression of apoA-I by transgenesis in mice and rabbits, which leads to a reduction in atherosclerosis, and the elimination of apoA-I expression in mouse models, which results in an accentuation of atherosclerosis. A few rare mutants of human apoA-I either enhance (ApoA-I_{Milano}) [5] or impair (ApoA-I_{Pisa} or ApoA-I_{FIN}) [6] the function of apoA-I and HDL. Indeed, apoA-I_{Milano} has been infused into patients to promote regression of atherosclerosis [7, 8]. However, recent GWAS studies that searched for loci influencing the development of CVD identified 46 relevant loci, but only about a third of these encompass known potential candidate genes [9]. For the assessment of the genetic control of HDL, HDL-C has largely been the measure employed. ApoA-I was not identified as a unique genetic risk factor [10].

This review will focus on studies that have examined genetic links between apoA-I/HDL and CVD in humans and in mice. A recent review comparing the results of human GWAS studies and genetic studies of atherosclerosis in mouse models demonstrated significant overlap in the risk factors for atherogenesis and genes influencing atherosclerosis in both species [11]**.

RELATIONSHIP OF APOA-I HERITABILITY TO ATHEROSCLEROSIS IN INBRED STRAINS OF MICE

The difficulty of obtaining a deep understanding of the mechanistic relationship of apoA-I/HDL to atherogenesis in humans encourages a turn to more readily manipulated experimental systems. Inbred strains of mice represent a very powerful resource to explore

questions about the loci that influence HDL and its atheroprotective function. Thirty years ago Beverly Paigen demonstrated the differential susceptibility to atherosclerosis of various inbred strains of mice [12, 13]. On the basis of such work, C57BL/6 mice have become the preferred inbred strain for research on atherosclerosis and HDL atheroprotective function. Two approaches have been adopted in the search for genes that might regulate HDL and its apoproteins as they influence atherogenesis. To obtain robustly measurable atherosclerotic lesions, genetically manipulated atherosclerosis susceptible models (e.g. *ApoE*^{-/-} and *Ldlr*^{-/-}) have been placed on atherosusceptible (e.g. C57BL/6) and atheroresistant (e.g. FVB, 129S6) genetic backgrounds and genes influencing atherosclerosis studied following intercrossing of the atherosusceptible and atheroresistant strains. Several QTL (quantitative trait loci) have been identified [14]. However, this approach has had limited success, largely due to the relative insensitivity in identifying specific candidate genes as each locus encompasses many potential genes. In one case, a strong atherosclerosis risk gene, *Raet1a*, encoding a histocompatibility class I protein was identified on chromosome 10b [15]. Of interest, none of the QTLs implicated a unique involvement of apoA-I in accounting for the differential sensitivity/resistance of the inbred strains of mice to atherosclerosis. A potential caveat is that most of these genetic studies have predominantly focused on measuring lesions in the proximal aorta, so involvement of the loci at other sites of lesion formation may yield different results. The importance of this caveat is exemplified by the finding of a QTL specific for aortic arch atherosclerosis in a cross of DBA/2J and strain 129 [16]. The aortic arch curvature and atherosclerosis QTL overlap [17].

A more highly resolving system has recently been developed to analyze genes influencing complex traits such as atherosclerosis and metabolic disorders. The Hybrid Mouse Diversity Panel (HMDP) is made up of 30 classical inbred strains + 70 or more recombinant inbred strains derived from crosses between C57BL/6 and DBA mice (BxD RI) and C57BL/6 and A/J mice (BxA and AxB RI) [18]**. The use of this panel of mice allows for high-resolution gene mapping that can be correlated with metabolic phenotype analysis and global gene expression in various tissues. Strains DBA and A/J are more resistant to atherosclerosis than C57BL/6. In order to screen for genetic loci influencing atherosusceptibility, each of HMDP strains were crossed with an atherogenic murine model that expresses two co-dominant transgenes, namely human *APOE-Leiden* and human *CETP*. This choice was made in place of the more frequently used atherosusceptible strains to avoid having to breed to the F2 generation to homozygosity for the null gene. In the studies of the HDMP, the heritability of HDL-C is relatively low [19]. In addition, the variation in HDL-C levels mapped to chromosome 5, which is not the locus of the apoA-I gene. Furthermore, in the analysis of 1,000 hepatic genes that differentially correlate with atherosclerosis in the proximal aorta, the expression of apoA-I does not appear. It is worth noting, however, that while the liver is the major site of apoA-I expression, the minor proportion of apoA-I synthesized in the intestine may contribute to HDL levels. This has yet to be systematically studied. Interestingly and parenthetically, there is no significant correlation of lipid loading of peritoneal macrophages with acetylated LDL *ex vivo* and the frequency of atherosclerotic lesions. ABCA1, a major cellular protein with which apoA-I interacts, appears low on the list of differentially expressed hepatic genes, but does not appear in the list of genes most differentially expressed in the aorta.

HERITABILITY OF HDL/APOA-I IN HUMAN POPULATIONS

Human and mouse HDL contain many closely related orthologous proteins, including apoA-I. Randomized clinical trials involving pharmaceutical targeting of HDL/apoA-I levels and Mendelian Randomization studies assessing if DNA variants are linked to atherosclerosis or CVD in large human groups have also failed to reveal a strong causal connection between coronary artery disease and HDL-C. Thus, both the murine inbred strains and the patient studies indicate that the strong negative epidemiological correlation of HDL (measured as level of HDL-C) with coronary artery disease is not of causal significance [20]**. This raises an important question. Is HDL-C the best indicator of HDL in atheroprotection? Identifying the best measure of HDL functionality with respect to atheroprotection is an active area of investigation [21]* and is addressed by Heinecke in this volume. Some investigators have studied the relative heritability of apoA-I instead of HDL-C. This may exhibit a better heritability index than does HDL-C [22]. Such a finding would be consistent with the preclinical studies in which the overexpression or elimination of apoA-I suggests significant involvement of apoA-I as a major atheroprotective agent, but studies involving a larger number of subjects are needed.

Recognizing the heterogeneity of HDL size and composition, it is possible that HDL as a cholesterol containing particle does not fully reflect the potential difference in the functional attributes of particle subspecies. ApoA-I is the major platform protein of HDL [23] and is required for the interaction with cell surface ABCA1 for the normal biogenesis of HDL. Thus, its absence or variants affecting its function results in a substantial decline in HDL levels, no matter how it is measured. These results taken together with human population studies and the murine HMDP studies suggest that variants in apoA-I are rare, even if these variants may have large effects on atherosclerosis and CVD risk in individuals with the genetic variant.

APOA-I IN THE FUNCTIONS OF HDL RELEVANT TO ATHEROGENESIS

The remainder of this review will discuss the functions of wild type apoA-I in inflammatory disorders or the function of genetic variants of human and murine apoA-I. Oxidatively modified apoA-I is much more highly enriched in atherosclerotic plaques than in the plasma, suggesting that the oxidative modifications occur locally in the vessel wall under the influence of the inflammatory cells of the evolving plaque. Much of this modification is mediated by myeloperoxidase. Oxidation of apoA-I may limit its capacity to afford atheroprotection. Oxidation of tryptophan 72, tyrosines 166 and 192 and methionine 148 all result in marked impairment of apoA-I's cholesterol efflux capacity [24–27] consequent on deficient interaction with ABCA1. The modified apoA-I is found in lipid poor particles. Furthermore, in the oxidative environment of the plaque, the malondialdehyde generated during the peroxidation of polyunsaturated fatty acids of phospholipids results in modification of the lysines of the two C- terminal helices of apoA-I, which also impairs cholesterol efflux [28]. The genetic determinants of this oxidative influence on apoA-I are not known.

ApoA-I_{Milano} is a major variant of human apoA-I that is due to the substitution of arginine 173 by cysteine. The identification of this variant excited much interest because of the relatively low incidence of CVD in families carrying this mutant protein, despite low levels of plasma HDL [29]. The atheroprotective influence of this variant was demonstrated in human patients infused with apoA-I_{Milano}-phospholipid complexes [30], although the precise mechanism of action is not clear. Studies on the expression of apoA-I_{Milano} in experimental models of atherosclerosis have not yielded consistent results. Using viral mediated hepatic expression of wild type and apoA-I_{Milano} in *Ldlr*^{-/-} mice, comparable reduction in atherosclerosis was observed [31]. Macrophage cholesterol efflux was also not different, though the Milano variant did not stimulate lecithin cholesterol acyl transferase activity as effectively as wild type apoA-I [32]. Yet, this latter impairment did not affect delivery of cholesterol to the liver. Viral mediated hepatic expression of apoA-I_{Milano} together with diet-mediated reduction of plasma lipid levels was more effective than lipid lowering alone in promoting regression of established lesions [33]*. On the other hand, the transplantation of bone marrow cells with retroviruses expressing apoA-I_{Milano} under the control of a macrophage specific promoter into mice deficient in apoA-I and apoE was more effective than wild type apoA-I expressing bone marrow cells in reducing lesions in the aorta, the aortic sinus and the innominate artery without changes in plasma lipid levels [34]. Both the Milano variant and the Paris variant (Arg¹⁵¹ to Cys), another naturally occurring variant associated with low HDL levels without increased CVD, inhibited the soybean lipoxygenase mediated lipid peroxide formation from unsaturated fatty acid suggesting enhanced anti-oxidative properties [35]. These studies suggest that as with HDL, it might be important to look for correlations of genetic variations with apoA-I function in addition to apoA-I levels.

We have recently reported on the capacity of HDL from 5 inbred strains of mice to promote cholesterol efflux [36]*. Using apoB depleted serum, serum HDL from each of the strains promoted efflux from J774 macrophages, but this was not correlated with the apoA-I content of the HDL. On the other hand, the ability of the isolated HDL from each of the strains to mediate efflux from BHK cell expressing ABCA1 was correlated with the apoA-I content. This suggests that ABCA1-independent pathways also participate in HDL mediated efflux. More than 30 years ago, differences in the charge of apoA-I was observed among 40 inbred strains of mice as assessed by isoelectric focusing [37]; 24 of the strains, including C57BL/6, exhibited one charge pattern, while 18 strains, including C3H, FVB and BALB/c (all resistant to the development of atherosclerosis) exhibited a different charge pattern. Sontag et al. analyzed functional differences in C57BL/6 and FVB/N HDL and apoproteins [38]. FVB/N mice have higher HDL levels, despite similar apoA-I levels. FVB/N HDL was larger and contained about two times as much apoA-II and more cholesteryl ester and phospholipid than C57BL/6 HDL. Sequence differences exist between the C57BL/6 and FVB/N apoproteins. In the case of apoA-I, glutamine 225 and valine 226 near the C-terminus of the protein were substituted by lysine and alanine respectively in FVB/N apoA-I. The cholesterol efflux capacity of the two apoA-I proteins did not differ significantly. On the other hand, the absorption of lipids and cholesterol from the intestinal lumen appears to contribute to the larger, cholesteryl ester rich HDL [39], with more cholesterol being absorbed by the small intestine of FVB/N mice. Finally, the enhancement of atherosclerosis

due to the absence of apoA-I in *ApoE*^{-/-} mice fed the western type diet in the two genetic backgrounds was investigated [40]. As expected, apoA-I deficiency in the C57BL/6 background resulted in an increase in atherosclerosis, despite a reduction in non-HDL lipoprotein cholesterol. This was not the case in the FVB/N background. The lesions were small in the FVB/N background even though the non-HDL cholesterol levels were higher than in the C57BL/6 mice and the absence of apoA-I had no effect on lesion size. We believe that the difference between the two strains rests upon the large difference in their respective non-HDL related atherogenic drive, rather than being a function of HDL/apoA-I. Contributions to this differential drive are not clear.

CONCLUSION

While epidemiological studies in human populations and preclinical studies in experimental animals provide strong evidence for the atheroprotective effect of apoA-I/HDL, studies in humans and mice looking for the association of genetic variants associated with apoA-I or HDL-C levels with CVD/atherosclerosis have not provided strong evidence for this atheroprotective function. Analysis of the association of genetic variants with functional attributes rather than levels may identify more informative loci.

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KEY POINTS

- Epidemiological studies in humans and preclinical studies in animal models provide strong evidence of the atheroprotective properties of apoA-I and HDL.
- However, GWAS in human populations and HDMP studies using inbred strains of mice have so far failed to link apoA-I to cardiovascular disease frequency, suggesting that genetic variants in structure and levels of apoA-I may play a very small role.
- The heritability of apoA-I/HDL in CVD protection in humans and in mice is low, suggesting that variations of apoA-I are not common. However, some rare apoA-I variants may have a large effect on cardiovascular disease in families.
- Additional GWAS and HMDP studies examining genetic links with HDL/apoA-I functions instead of levels may identify new loci.