Interaction Between a Polymorphism of the Apo A-I Promoter Region and Smoking Determines Plasma Levels of HDL and Apo A-I

Gardar Sigurdsson Jr., Vilmundur Gudnason, Gunnar Sigurdsson, and Steve E. Humphries

Epidemiological studies have demonstrated that the risk of coronary artery disease (CAD) is increased in smokers but reduced in individuals with elevated plasma levels of high density lipoprotein cholesterol (HDL-C) and apolipoprotein (apo) A-I. In a sample of 315 men and women from Iceland, the mean levels of HDL-C and apo A-I in smokers were, respectively, 12% and 6% lower in men and 7% and 6% lower in women compared with nonsmokers. In the men who were nonsmokers, a guanine (G) to adenine (A) (G/A) substitution 75 bp upstream from the start of transcription of the apo A-I gene was associated with elevated levels of HDL-C and apo A-I, with those carrying the A allele having levels of HDL-C and apo A-I roughly 10% higher than those with only the G allele. This genotype effect was abolished in the men who smoked and absent in the sample of women. Based on the reported protective effect associated with elevated levels of apo A-I and HDL-C, men carrying the A allele would have roughly a 20% lower relative risk of CAD compared with those without the A allele, but only if they remained nonsmokers. This gene polymorphism would therefore be an important factor in determining those men who would most benefit from avoidance or cessation of smoking. Determining the mechanism of such interaction between genotype and environment will be important in understanding the etiology of CAD.

KEY WORDS • apolipoprotein A-I • high density lipoproteins • promoter • gene polymorphism • smoking • gene-environment interaction

A polipoprotein (apo) A-I is the major protein constituent of high density lipoprotein (HDL) particles and plays a crucial role in lipid transport and metabolism. Several epidemiological studies have reported that HDL cholesterol (HDL-C) and apo A-I are inversely related to the incidence1-3 and severity4-5 of coronary artery disease (CAD) and can independently predict the risk of CAD.2,3 Apo A-I is the major in vivo activator of lecithin: cholesterol acyltransferase,6 appears to promote cholesterol efflux from cells,7,8 and is therefore an important component of "reverse cholesterol transport."9 Levels of HDL-C and apo A-I are known to be influenced by a number of factors, including diet, exercise, sex hormones, alcohol intake, age, and body mass index (BMI).10-15 Cigarette smoking is well recognized as a risk factor of CAD16 and has been shown to have a lowering effect on the levels of both HDL-C and apo A-I.17-19 This effect is reversible, and within 2 weeks from cessation of smoking the levels of HDL-C return to normal.20,21

A strong genetic effect on the levels of HDL-C and apo A-I has also been demonstrated by twin and family studies.22,23 The gene for apo A-I is known to be in a cluster with apo C-III and apo A-IV on chromosome 11,24 and several studies have reported an association between DNA polymorphisms of this gene cluster and differences in the levels of HDL-C and apo A-I in CAD patients and healthy individuals.25-27 Recently, a guanine (G) to adenine (A) (G/A) substitution has been described that is 75 bp from the start of transcription of the apo A-I gene.28,29 This substitution destroys an Msp I cutting site and can be detected easily with the polymerase chain reaction (PCR). In three independent studies, the A allele has been associated with higher levels of both HDL-C and apo A-I.28-30 In this study of individuals from the Icelandic population, we show that this polymorphism is associated with differences in levels of apo A-I and HDL-C in men but not in women and that the potential beneficial effect on CAD risk, which may result from inheritance of the A allele of this gene polymorphism, is completely abolished in those men who smoke.

Methods

The subjects, 149 men and 166 women aged 15-78 years old, were chosen from randomly selected participants in the Icelandic National Diet Survey 1990. All subjects came from the southwestern part of Iceland.
and the greatest proportion were from the capital, Reykjavik. All subjects completed a questionnaire concerning smoking habits. In this study we defined smokers as all those who currently smoked tobacco or had ceased smoking up to 14 days before blood sampling. Those taking lipid-lowering drugs or thyrroxin or with diabetes were excluded. Blood samples were collected at the Icelandic Heart Association Research Centre after an overnight fast. Total serum cholesterol (TC) and serum triglyceride (TG) concentrations were measured by automated enzymatic colorimetry (Cobas Mira, Roche). HDL-C was measured enzymatically after phosphotungsten/magnesium precipitation. Both internal and external laboratory controls were used. Low density lipoprotein cholesterol (LDL-C) was estimated by the Friedewald formula (Friedewald et al.12). Apo A-I was measured with an automated turbidimetric method (Cobas Mira, Roche MA 30). The apo A-I measurements were done on serum that had been stored at -20°C for 1-5 months. No significant correlation was found between time of storage and levels of the apoproteins. The overall coefficient of variation (CV) for apo A-I was 3.4%, and the intra-assay and interassay CVs were 1.8% and 1.9%, respectively.

DNA was extracted from 10 ml of EDTA-containing blood by the salting-out method. Amplification of a 432-bp region of the apo A-I gene and promoter was done by the PCR method34 by using 250 ng of each of the two primers, P1 (5'-AGGGGACAGAGCT-GATCCTCTGA3') and P2 (5'-TTAGGGGACACCTAGGCTCGAGGAAGCA3'), with 200 ng genomic DNA and 0.1 unit Thermus aquaticus DNA polymerase (Bethesda Research Laboratories, Gaithersburg, Md.) in a total volume of 50 µl with the buffer recommended by the manufacturer. The reactions were performed on a Cambio 'intelligent thermal cycler' (Cambio Limited, Cambridge, UK) in a total volume of 35 µl with the buffer recommended by the manufacturer. The DNA fragments were separated by electrophoresis on a 7.5% polyacrylamide gel with 2% urea at 120 V for 2 hours. The gel was then stained with ethidium bromide and photographed under UV light.

Statistical analysis was performed by using the software package SPSS/PC+. Statistical significance was considered to be at the 0.05 level. Student's t test was used for the comparison of each lipid trait, BMI (weight in kilograms divided by the square of height in meters), and age between genders and between smokers and nonsmokers. In both men and women, there was a correlation (p<0.01) between BMI and HDL-C (r= -0.26 and -0.24, respectively), and in women there was a correlation (p<0.01) between age and apo A-I (r=0.18). Stepwise regression was used to adjust HDL-C and apo A-I for age and BMI. χ² Analysis was used to test for Hardy-Weinberg equilibrium. There were only four individuals of genotype AA (two men and two women), and in the calculation they were included in the GA group. Analysis of variance (ANOVA) was performed to compare the mean levels of lipid param-

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>149</td>
<td>166</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.1 (1.2)</td>
<td>42.6 (1.2)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.1 (0.5)</td>
<td>24.1 (0.3)</td>
</tr>
<tr>
<td>TC (mmol/l)</td>
<td>5.68 (0.09)</td>
<td>5.51 (0.09)</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>1.55 (0.05)</td>
<td>0.98 (0.04)*</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.16 (0.02)</td>
<td>1.44 (0.03)†</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.89 (0.08)</td>
<td>3.63 (0.09)*</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>144.8 (1.7)</td>
<td>162.1 (1.9)†</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>30.7</td>
<td>35.3</td>
</tr>
</tbody>
</table>

BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; apo, apolipoprotein. Values are mean and (SEM).

*p<0.05, †p<0.01.

The characteristics of the individuals studied are shown in Table 1. As expected, there are significant differences between men and women for levels of TG, LDL-C, HDL-C, and apo A-I. In both men and women, lower levels of HDL-C and apo A-I were observed in those who smoked compared with those who did not (Table 2). No significant difference was found in levels of HDL-C and apo A-I between those who had never smoked and those who were former smokers (data not shown).

The G/A substitution destroys an Msp I cutting site, and genotype was determined in all samples by polyacrylamide gel electrophoresis of an Msp I cut amplified fragment of the apo A-I promoter (Figure 1). The frequency of the A allele was 0.12 in both men and women. The distribution of genotypes in this sample was not significantly different from that expected of a sample in Hardy-Weinberg equilibrium.

Because of differences in the levels of HDL-C and apo A-I between men and women and between smokers and nonsmokers, the association between genotype and lipid traits was analyzed separately in the four groups. Results are presented in Table 2 and graphically in Figure 2. The G/A substitution was only significantly associated with differences in the levels of HDL-C and apo A-I in nonsmoking men. Those who had the A allele had 11% higher levels of HDL-C and 9.7% higher levels of apo A-I, and after adjustment for age and BMI, the percentage of sample variance (R²×100) accounted for by genotype was 13.9% and 8%, respectively (Table 2). Figure 2 shows the percent difference of adjusted HDL-C and apo A-I levels from the mean of the sample in the groups of men with different genotypes and smoking habits. There is a 15% difference in apo A-I levels and a 20% difference in HDL-C levels between smokers and nonsmokers in those with one or more A alleles. In those with only the G allele, the difference

Table 1. Characteristics of the Sample of Men and Women From the General Population of Iceland
TABLE 2. Adjusted Levels of HDL-C and Apo A-I in Men and Women With Different Apo A-I G/A Genotypes in Smokers and Nonsmokers

<table>
<thead>
<tr>
<th>Genotype group</th>
<th>Nonsmokers</th>
<th>Smokers</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>n=104</td>
<td>n=45</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.21(0.03)</td>
<td>1.06(0.03)</td>
<td>p=0.002</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>147.2(2.2)</td>
<td>138.9(2.5)</td>
<td>p=0.023</td>
</tr>
<tr>
<td>GG</td>
<td>n=76</td>
<td>n=34</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.17(0.03)</td>
<td>1.07(0.04)</td>
<td>p=0.032</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>143.5(2.4)</td>
<td>140.4(2.8)</td>
<td>NS</td>
</tr>
<tr>
<td>GA+AA</td>
<td>n=28</td>
<td>n=11</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.31(0.07)*</td>
<td>1.04(0.07)</td>
<td>p=0.023</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>157.4(4.4)*</td>
<td>134.4(5.4)</td>
<td>p=0.005</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>n=107</td>
<td>n=59</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.48(0.03)</td>
<td>1.37(0.04)</td>
<td>p=0.028</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>165.7(2.4)</td>
<td>155.9(2.8)</td>
<td>p=0.011</td>
</tr>
<tr>
<td>GG</td>
<td>n=87</td>
<td>n=44</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.49(0.04)</td>
<td>1.35(0.04)</td>
<td>p=0.024</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>166.4(2.7)</td>
<td>156.1(3.4)</td>
<td>p=0.024</td>
</tr>
<tr>
<td>GA+AA</td>
<td>n=20</td>
<td>n=15</td>
<td></td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.43(0.09)</td>
<td>1.40(0.07)</td>
<td>NS</td>
</tr>
<tr>
<td>Apo A-I (mg/dl)</td>
<td>162.9(5.2)</td>
<td>155.5(4.5)</td>
<td>NS</td>
</tr>
</tbody>
</table>

HDL-C, high density lipoprotein cholesterol; apo, apolipoprotein; G/A, guanine to adenine; NS, not significant; ANOVA, analysis of variance. Values are mean and (SEM).

*p=0.029, difference in mean levels of HDL-C between genotypes in nonsmokers by ANOVA.

p=0.004, difference in mean levels of apo A-I between genotypes in nonsmokers by ANOVA.

between smokers and nonsmokers only reached statistical significance for HDL-C, although the trend can be seen for apo A-I as well.

To test for interaction between genotype, gender, and smoking, a two-way ANOVA was performed. For apo A-I, significant interaction (p<0.02) was found between genotype and gender in nonsmokers and between genotype and smoking in men (p<0.02). For HDL-C, a similar trend was seen for the interaction between gender, smoking, and genotype, but these differences did not reach statistical significance. In this sample there was the expected inverse relation between triglyceride levels and HDL and apo A-I concentrations; however, there were no significant differences in the mean triglyceride levels, age, or BMI between genotypes in either smokers or nonsmokers. For the non-smoking men, the mean triglyceride levels were 1.19 mmol/l and 1.07 mmol/l for those with and without the A allele, respectively. There was no significant difference in the levels of other lipid parameters between genotypes of the apo A-I gene in either men or women (data not shown).

Discussion

It is well known that both environmental11,15,35 and genetic22,23,34 factors contribute to the determination of plasma levels of HDL-C and apo A-I. The apo A-I gene itself is an obvious candidate for study, and in recent years restriction fragment length polymorphisms have been used to demonstrate that genetic variation in the apo A-I-C-III-A-IV gene region partly determines the levels of apo A-I in healthy individuals and patients.25-27 The G/A substitution at −75 bp upstream from the start of transcription of the apo A-I gene has been shown in two previous studies from our laboratory to be strongly associated with higher levels of HDL-C and apo A-I in healthy males.28,29 Similar observations have also been reported in a study of individuals from Italy, although in contradiction to the observations on the individuals from Iceland, Pagani et al29 reported a stronger association in women than in men. However, in that study, levels of apo A-I were not measured, and no clear distinction was made between smokers and nonsmokers. It is well known that there is an inverse relation between triglyceride levels and HDL-C and apo A-I levels, and this trend was observed in this sample. However, mean TG levels were similar in nonsmoking men with different G/A genotypes, which thus cannot explain the association seen. In the data we present here, there was evidence for significant interaction between gender and genotype, with the A allele being associated with elevated levels of HDL-C and apo A-I.
Figure 1. Panel a: Pattern observed when polymerase chain reaction and polyacrylamide gel electrophoresis are used to reveal genotypes after Msp I digestion of the fragment shown schematically in panel b. Lanes 2 and 3 show individuals homozygous for the G allele (cutting), lane 1 shows an individual heterozygous for the cutting site, and lane 4 shows an individual homozygous for the A allele (noncutting). In panel b, Msp I cutting sites are indicated by transverse bars, and their positions are shown relative to the transcription initiation site, as are the relative positions of the CAT and TATA boxes that flank the guanine to adenine (G/A) substitution. All measurements are in base pairs.

Only in men. The difference in response to the G/A substitution between men and women could be through the effect of sex hormones because levels of HDL-C and apo A-I are positively associated with endogenous levels of testosterone in adult men.12 Because of its position in the promoter region of the apo A-I gene, it is likely that the G/A sequence change itself may have a direct effect on the transcription of the apo A-I gene and thereby the secretion of apo A-I from either the liver or the intestine. A recent study using DNase I footprint analysis on the proximal part of the apo A-I promoter has identified four protected areas,37 with one of the protein binding regions being from -128 bp to -77 bp. This is close to the G/A sequence change at -75 bp, and our hypothesis is that the G/A substitution alters the affinity for a transacting nuclear protein that affects the transcription rate of the apo A-I gene and thus alters the rate of synthesis of apo A-I from the liver or intestine. One possibility is that testosterone may increase the level or affinity of one of the nuclear proteins and would thus affect synthesis and secretion of apo A-I in an allele-specific manner. Functional tests of promoter strength are in progress in our laboratory to examine this hypothesis.

In this sample of nonsmoking men, the G/A polymorphism explained more than 8% of the variance in apo A-I levels and more than 13% of HDL-C levels. Because of the well-established effect on reduced risk of CAD associated with elevated HDL-C and apo A-I levels, this polymorphism can be expected to be one of those contributing to an individual’s risk of CAD through an effect on reverse cholesterol transport.9 In this sample of individuals from Iceland, >30% reported that they were current smokers, which is similar to that reported in a recent large Icelandic population study,38 and both HDL-C and apo A-I levels are lower in male and female smokers, confirming the results of others.13,14 The mechanism of this smoking effect is unknown, but apo A-I is a negative acute-phase protein,39,40 and the action of growth factors such as transforming growth factor-β on gene expression in the liver may explain this effect, possibly acting through posttranscriptional mechanisms affecting mRNA stability.41

Several studies have shown evidence for an interaction between genotype and smoking in the determination of apo A-I and HDL-C levels.42,43 Our data in the men provide evidence for significant interaction between the G/A genotype and smoking in the determination of these traits. These observations need to be confirmed by other studies, but similar observations have been made in our laboratory in a group of healthy Chinese men (H. Paul-Hayase et al, unpublished observations). The data suggest that by some mechanism, smoking overrides the positive effect of the A allele on levels of apo A-I and HDL-C, with the result that men carrying the A allele would be predicted to change from having some of the highest levels of HDL-C and apo A-I to some of the lowest levels if they are smokers. The effect of this interaction on the relative risk of CAD can be estimated by extrapolation from a recent Icelandic study, which showed that a 1% change in apo A-I level was associated with a 2% change in the relative risk of CAD.44 Based on these data, which are consistent with other recent studies,22 those men with an A allele would have a risk of CAD roughly 80% that of individuals who lack the A allele, but if these same individuals smoke cigarettes, their relative risk will increase by 1.3-fold.
because of the large (15%) decrease in levels of apo A-I and will also increase by 1.8-fold because of the direct effect associated with smoking, for a total increase in relative risk of 2.3-fold. Similar estimates were obtained with HDL-C as a predictor of relative risk.2,3

Understanding the mechanism of the interaction between this gene polymorphism and environmental factors will be important in determining the etiology of low plasma HDL-C and apo A-I levels and, in particular, in identifying those smoking individuals who would lower their risk of CAD substantially by cessation of smoking.

Acknowledgments
We thank the staff of the Icelandic Heart Association Research Center and L. Steingrimsdottir for their assistance.

References
13. Criqui NH, Wallace RB, Heiss G, Mishkel M, Schonfeld G, Jones GTL: Cigarette smoking and plasma high-density lipoprotein cho-

17. Moll PP, Michels VV, Weidman WH, Kotake BA: Genetic determina-
tion of plasma apolipoprotein AI in a population-based sam-
21. Wife DB, Barbir M, Gallagher J, Myant NB, Ritchie CD, Thomp-
24. Paul-Haye H, Rossenau M, Robinson D, Van Bervliet JP, Deslyp-
25. Lopes-Virella MF, Stone P, Ellis S, Cotwell JA: Cholesterol deter-
26. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the con-
28. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the con-
32. Haffner SM, Applebaum-Bowden D, Wahl PW, Hoover JJ, War-
36. Sigurdsson et al. Apo A-I Gene Promoter and 1021

Apo


Interaction between a polymorphism of the apo A-I promoter region and smoking determines plasma levels of HDL and apo A-I.

G Sigurdsson, Jr, V Gudnason, G Sigurdsson and S E Humphries

doi: 10.1161/01.ATV.12.9.1017

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/12/9/1017

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/