Atherosclerosis and Lipoprotein

Genetic Contributions to Plasma Total Antioxidant Activity
Xing Li Wang, David L. Rainwater, Jane F. VandeBerg, Braxton D. Mitchell, Michael C. Mahaney

Abstract—Oxidative stress plays important roles in a wide spectrum of pathological processes, such as atherosclerosis. Although several environmental factors are documented to influence redox metabolism, relatively little is known about genetic effects. In the present study, we evaluated genetic contributions to variation in plasma total antioxidant status (TAS), a measure of peroxyl-scavenging capacity, in 1337 members of 40 Mexican American families. TAS levels were significantly lower in women than in men (1.675 ± 0.004 versus 1.805 ± 0.005 mmol/L, respectively; \( P < 0.001 \)), and there was a significant decline of TAS levels with age in men but not in women \(( P < 0.01 \) for the interaction). Quantitative genetic analysis indicated the heritability of TAS levels to be 0.509 ± 0.052; ie, \( ~ 
\) 51% of the residual variance (after covariate adjustment) in TAS levels was due to the additive effects of genes \(( P < 0.001 \)). We have further observed a significant gene-by-smoking interaction \(( P < 0.05 \)). Additive genetic effects account for 83% of the residual phenotypic variance in TAS levels among smokers, but they account for only 49% in nonsmokers. However, genes contributing to TAS variation are the same in smokers and nonsmokers. Our study for the first time demonstrates that TAS, an indicator of redox homeostasis, is under strong genetic control, especially among smokers. With appropriate tools, such as genome screening, it should be possible to localize genes that regulate redox homeostasis and, ultimately, identify the DNA sequence variants predisposing subjects to oxidative damage. (Arterioscler Thromb Vasc Biol. 2001; 21:1190-1195.)

Key Words: antioxidants ■ coronary disease ■ genetics ■ statistics ■ smoking

All aerobic organisms, including humans, derive most of their metabolic energy from the reduction of oxygen and, consequently, are susceptible to the damaging effects of the small amounts of \( O_2^- \), \( \cdot OH \), and \( \cdot H_2O_2 \) that are produced during the metabolism of oxygen. These 3 species, together with unstable intermediates in the peroxidation of lipids, are referred to as reactive oxygen species (ROS). ROS can react with nearly all biological macromolecules (lipids, proteins, nucleic acids, and carbohydrates). The initial reaction generates a second radical, which can, in turn, react with a second macromolecule to continue the chain reaction. As protection against the deleterious effects of free radicals, the human body has developed an antioxidant defense system that includes enzymatic, metal-chelating, and free radical–scavenging activities. They include superoxide dismutases (SODs), catalase, glutathione peroxidase, glutathione reductase, vitamins C and E, \( \beta \)-carotene, and lipoic acid.1,2

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Many diseases, such as atherosclerosis, appear to result from an overbalance between radical-generating, compared with radical-scavenging, systems, a condition called oxidative stress. In addition, oxidative stress has been proposed to be a major mechanism that accelerates the aging process,3 and various antioxidants have been tested as antiaging therapies in humans and in animal models with mixed results.4 Hyperglycemia or insulin-related metabolic disturbances in diabetes can induce excessive oxidative stress in type 1 and 2 diabetes.5,6 Neurodegenerative disorders, including Alzheimer’s disease, Parkinson’s disease, corticobasal degeneration, memory losses, Pick’s disease, and various dementias, are also associated with increased oxidative stress.7–9 Finally, ROS are a direct cause of DNA damage, which is often the trigger for carcinogenesis.

A number of mechanisms, which are not mutually exclusive, have been proposed to explain a possible connection between oxidative stress and coronary artery disease. ROS can damage endothelial cells in many ways, either directly or indirectly.10 ROS can promote endothelial apoptosis, leading to an increased tendency toward thrombosis and endothelial dysfunction.11 They can also increase endothelial permeability and thereby accelerate the accumulation of atherogenic factors, such as LDL, in the subendothelial cell space. They stimulate endothelial cell production of many adhesion molecules, so the vascular wall becomes prothrombotic and proatherogenic.12 Oxidative stress is also associated with stimulation of vascular smooth muscle cell apoptosis and necrosis and contributes to the formation of the necrotic core, a hallmark of an advanced unstable lesion. Therefore, ROS have been implicated to play a strong role in atherogenesis. However, clinical trials using antioxidants, such as vitamins C and E and \( \beta \)-carotene, have produced conflicting re-
sults. This may reflect complex balanced chain reactions of oxidants and antioxidants in the redox system. It may also reflect a lack of appreciation of the specificity of oxidative stress and responses to antioxidants and the importance of the subcellular environment.

Although dietary factors play significant roles in ROS production, genetic factors may also contribute to its bioregulation. Liao et al have reported evidence of a common genetic pathway mediating oxidative stress–induced inflammatory gene expression, and they have suggested a major contributing gene. A G→A substitution at the fifth position of intron 4 of the catalase gene was found to be responsible for acatalasia in Japanese individuals. Heterozygotes have an intermediate level of catalase in the blood, and patients are more likely to have infective diseases. Mutations occurring at the SOD1 gene are associated with reduced SOD activities and amyotrophic lateral sclerosis. A DNA variant at the SOD3 gene is associated with a reduced affinity for heparin, which may compromise the ability of SOD3 to bind to the vascular wall, thereby reducing its antioxidant capacity. Moreover, we have reported significant genetic contributions to the variance of common SOD3 phenotypic traits. However, these genetic contributions are mostly confined to the known candidate enzymes. The frequencies of these mutations are too rare to account for population variances in ROS-related common diseases, such as atherosclerosis.

We have begun a systematic study of the genetic control of oxidative stress, focusing on Mexican Americans in San Antonio, Tex. In the present study, we have explored genetic contributions to a global measure of total antioxidant status (TAS) in human plasma. TAS reflects the balance between antioxidants and oxidants in each system. Whereas the ROS-producing enzymes determine how many free radicals are produced, the antioxidant system determines whether these ROS are in excess, which classes of molecules they will oxidize, and what pathological changes they will leave behind. We hypothesize that oxidative stress and its subsequent pathological processes are under significant genetic regulation. Dissecting genetic contributions to TAS will lead us to discover potentially novel genes that contribute to both sides of the balance and that are critical to clinical or pathological outcomes.

Methods

Subjects and Blood Samples

Samples were provided by participants in the San Antonio Family Heart Study (SAFHS), a study of the risk factors for cardiovascular disease in Mexican Americans. All first-, second-, and third-degree relatives of randomly ascertained probands were invited to participate, and the present study group was composed of 1337 individuals in 40 families. At the clinic visit, participants were subjected to an oral glucose tolerance test, and they provided information on health and lifestyle variables and a fasting blood sample. Plasma was isolated by low-speed centrifugation and was stored as single-use aliquots at −80°C, which were protected from oxidation and desiccation. Clinic procedures were approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio, and subjects gave written informed consent.

Covariates

Diabetes status (yes/no) was diagnosed by World Health Organization criteria or by whether the subject was currently taking medication for diabetes. Information on age, menopausal status, current smoking status, and medication usage was obtained during the clinic interview.

Measurement of TAS

TAS levels reflect the overall antioxidant capacity of the human plasma and summarize the pertinent physiological and pathological conditions at the time of sample collection. The assay was defined as the ability of antioxidants in the plasma samples to prevent oxidation of 2,2′-azino-di-(3-ethylbenzthiazoline sulfonate) (ABTS) by metmyoglobin and was quantified by using a commercial kit (Total Antioxidant Status Assay Kit, Calbiochem) that was based on the method of Miller et al. Antioxidant ability of the sample was expressed relative to the standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in millimolar units. The interassay and intra-assay coefficients of variation were 2.0% and 1.3%, respectively.

Statistical Genetic Analyses

In our analyses, we assume that variation in TAS levels is jointly influenced by genes and the environment. We use a variance decomposition approach, implemented in the statistical genetic analysis software package SOLAR (Sequential Oligogenic Linkage Analysis Routines), to estimate the effects of genes, selected environmental covariates, and unmeasured nongenetic factors on the variance in TAS levels. This approach, developed according to methodology originally proposed by Hopper and Mathews and Boehnke et al, has been described in detail elsewhere. It models the phenotypic covariance (ie, ) as the sum of the covariance due to the additive effects of genes (ie, ), where is the matrix of kinship coefficients between all relative pairs in a pedigree and and are the additive genetic variance and the environment (ie, ), where is an identity matrix with all diagonal elements equal to 1.0 and off-diagonal elements equal to 0 and is the variance due to unmeasured environmental factors) and allows us to partition the phenotypic variance in TAS levels ( where subscript indicates phenotype) into components corresponding to the additive genetic effects ( ) and nongenetic (ie, environmental) effects ( ). Like the components of the covariance, these variance components are additive, such that and and we estimate the heritability (h²), or proportion of the phenotypic variance attributable to additive genetic effects, as and the proportion attributable to nongenetic factors as .

We conducted initial statistical genetic analyses to detect and measure the effects of genes and nongenetic factors on the variance in TAS levels. To accomplish this, we simultaneously estimated the values of the phenotypic mean±SD and h², as well as the mean effects of age and sex terms. We also screened a number of selected environmental covariates for inclusion in this model. These included alcoholic beverage ingestion, diabetes, antidiabetic medication use, lipid-lowering medication use, hormone replacement therapy, oral contraceptive use, menopausal status, and cigarette smoking.

Multivariate extensions to the basic quantitative genetic analyses used to detect and measure the effects of genes and significant environmental covariates were used to test also for the effects of genotype-by-covariate interactions on variance in the TAS levels. In the absence of genotype-by-environment interaction (ie, the null hypothesis), the genetic correlation between relatives for a trait measured under 2 environments should be and the genetic variances in the 2 environments should be equal (ie, ). Rejection of the former hypothesis (ie, ) would imply that a different gene or suite of genes is contributing to the variance in the TAS levels in both environments, whereas rejection of the latter (ie, ) would imply that the magnitude of the genetic effect is different in the 2 environments.

Parameter estimates and their standard errors are obtained by numerical maximization of the likelihood of models on data from relatives in the SAFHS pedigrees. Statistical significance of each parameter is assessed by means of likelihood ratio tests, in which the likelihood of a general unrestricted model wherein the values for all parameters are estimated is compared with the likelihood of
altimate restricted models (hypotheses) in which the values for parameters of interest are constrained to some predetermined value (eg, 0 or 1). Obtaining degrees of freedom for specific tests is explained in detail elsewhere.25,26 Although P=0.10 served as the covariate inclusion criterion during the initial screen, P=0.05 was required to declare a genetic or genotype-by-environment effect to be significant.

Results

Effects of Age and Sex on TAS

The TAS levels in the total population appeared to be normally distributed (Figure 1), and the mean±SEM value of TAS in this Mexican American population was 1.728±0.004 mmol/L. Table 1 gives some of the characteristics of the population and indicates that TAS levels were higher in men than in women (1.805±0.005 versus 1.675±0.004 mmol/L, respectively; P<0.001). In addition, there were significant effects of age in men. In men, mean TAS levels decreased with increasing age, whereas TAS levels remained relatively constant across age groups in women (Figure 2). A formal test for statistical interaction revealed that the effect of age on TAS levels was significantly greater in men than in women (P<0.01).

Quantitative Genetic Analysis of TAS Levels

Table 2 gives the results of quantitative genetic analyses, which included several covariates (diabetes status, diabetes medications, contraceptive hormone use, menopausal status, and smoking status), in addition to age and sex. Normal quantitative variation in TAS levels was moderately heritable in the SAFHS pedigrees (Table 2). Seven variables, including sex, age, age², age-by-sex interaction, menopausal status, oral contraceptive use, and smoking were identified by our initial screening procedures as likely covariates of TAS level in these subjects (P≤0.10). Together, these covariates accounted for ≈25.8% of the total phenotypic variance in TAS levels. Of the remaining 74.2%, the additive effects of genes accounted for 50.9% (37.8% of the total phenotypic variance), and 49.1% (36.4% of the total variance) was due to random environmental (ie, unmeasured nonadditive genetic) effects.

Effects of Diabetes on TAS

Diabetes medication usage, but not diabetes status, was a significant predictor of TAS variation. We hypothesized that diabetes medication usage might be a surrogate indicator of diabetes severity and that 2 other indicators of diabetes severity (diabetes duration and fasting glucose levels) would be correlated with TAS levels in the diabetic subjects (n=195). However, neither diabetes duration nor fasting glucose level was a significant predictor of TAS variation in diabetic patients (P=1 and P=0.69, respectively) nor was the

**TABLE 1. Characteristics of Study Group According to Sex**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Women (n=789)</th>
<th>Men (n=548)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>39.6±0.6</td>
<td>38.6±0.7</td>
</tr>
<tr>
<td>Smoking, %</td>
<td>16.0</td>
<td>34.9</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>15.1</td>
<td>13.9</td>
</tr>
<tr>
<td>Diabetes medications, %</td>
<td>8.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Oral contraceptives, %</td>
<td>10.6</td>
<td>...</td>
</tr>
<tr>
<td>Menopause, %</td>
<td>22.7</td>
<td>...</td>
</tr>
<tr>
<td>TAS, mmol/L</td>
<td>1.675±0.004</td>
<td>1.805±0.005</td>
</tr>
</tbody>
</table>

**TABLE 2. Genetic and Environmental Effects on Variation in TAS in 1337 Mexican Americans: MLE of Mean, Coefficient (β) for Covariates, and Proportion of Variance Attributable to Additive Genetic and Nongenetic Effects**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MLE±SE</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>18.05±0.66</td>
<td>...</td>
</tr>
<tr>
<td>β value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>−1.27±0.07</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age</td>
<td>−0.014±0.003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age×sex</td>
<td>0.011±0.004</td>
<td>0.0484</td>
</tr>
<tr>
<td>Age²</td>
<td>−0.0002±0.0001</td>
<td>0.0532</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.079±0.052</td>
<td>0.533</td>
</tr>
<tr>
<td>Diabetes medication</td>
<td>−0.314±0.151</td>
<td>0.0390</td>
</tr>
<tr>
<td>Menopause</td>
<td>0.253±0.128</td>
<td>0.0816</td>
</tr>
<tr>
<td>Oral contraceptive use</td>
<td>−0.389±0.127</td>
<td>0.0022</td>
</tr>
<tr>
<td>Smoking</td>
<td>0.137±0.072</td>
<td>0.0792</td>
</tr>
<tr>
<td>h²†</td>
<td>0.5089±0.0518</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>e²†</td>
<td>0.4911±0.0518</td>
<td>...</td>
</tr>
</tbody>
</table>

MLE indicates maximum likelihood estimate.

*Nested model in which the covariate was fixed at 0.
†Additive effects of genes (h²) and nongenetic factors (e²) expressed as a proportion of the residual phenotypic variance in TAS levels (ie, that remaining after accounting for proportion of phenotypic variance due to covariate effects, or 25.8%).

![Figure 1. Frequency histogram for the distribution of TAS levels in study subjects (n=1337).](image1)

![Figure 2. Changes in TAS (mean±2 SEM) in men (open circles) and women (closed circles) with age. Age was arbitrarily divided into 10-year intervals.](image2)
fasting glucose level a significant predictor of TAS variation in the entire population \( (P=0.62) \).

**Effects of Cigarette Smoking on TAS Levels**

The results of tests for gene-by-smoking effects and covariate-by-smoking effects on variation in TAS levels in the SAFHS pedigrees are summarized in Table 3. Covariates included in Table 3 are those satisfying the \( P \leq 0.1 \) criterion. Likelihood ratio tests again revealed significant effects \( (P \leq 0.03) \) of sex, age, age\(^2\), menopausal status, and oral contraceptive use on variation in TAS levels. They also provided strong evidence \( (P \leq 0.002) \) of interactions between cigarette smoking and the following variables: age, age-by-sex, and diabetic medication use; and they provided tentative evidence \( (P=0.0672) \) of a smoking-by-diabetes effect on TAS levels.

A genotype-by-smoking effect on normal variation in TAS levels was detected also. The genetic variance in smokers was significantly greater than that in nonsmokers \( \left( \text{h}^2 = 0.452 \right) \). Consequently, additive genetic effects accounted for a much greater proportion of residual phenotypic variance in smokers versus nonsmokers (ie, \( \text{h}^2 \) was 83% and 49%, respectively).

Although the maximum likelihood estimate for the genetic correlation between smokers and nonsmokers did not equal 1.0, it was high \( \left( \rho_6 = 0.798 \right) \) and not significantly different from unity at the \( P=0.05 \) level \( (P=0.09) \). This suggests that most, if not all, of the genetic effects on variation in TAS levels in nonsmokers and smokers is due the same gene or suite of genes.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MLE±SE</th>
<th>Hypothesis</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>18.061±0.067</td>
<td>( \beta ) sex = 0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( \beta ) sex</td>
<td>-1.271±0.068</td>
<td>( \beta ) age = 0</td>
<td>0.0049</td>
</tr>
<tr>
<td>( \beta ) age</td>
<td>-0.007±0.002</td>
<td>( \beta ) age(^2) = 0</td>
<td>0.0305</td>
</tr>
<tr>
<td>( \beta ) age(^2)</td>
<td>-0.0002±0.0001</td>
<td>( \beta ) menopause = 0</td>
<td>0.0074</td>
</tr>
<tr>
<td>( \beta ) menopause</td>
<td>0.297±0.111</td>
<td>( \beta ) oral contraceptive use = 0</td>
<td>0.0014</td>
</tr>
<tr>
<td>( \beta ) age×smoking</td>
<td>-0.019±0.005</td>
<td>( \beta ) age×smoking = 0</td>
<td>0.0005</td>
</tr>
<tr>
<td>( \beta ) age×sex×smoking</td>
<td>0.025±0.008</td>
<td>( \beta ) age×sex×smoking = 0</td>
<td>0.0022</td>
</tr>
<tr>
<td>( \beta ) diabetes×smoking</td>
<td>0.452±0.247</td>
<td>( \beta ) diabetes×smoking = 0</td>
<td>0.0672</td>
</tr>
<tr>
<td>( \beta ) diabetes medication×smoking</td>
<td>-0.988±0.316</td>
<td>( \beta ) diabetes medication×smoking = 0</td>
<td>0.0020</td>
</tr>
<tr>
<td>( \alpha_s ) smokers</td>
<td>1.052±0.116</td>
<td>( \alpha_s ) smokers = 0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( \alpha_s ) nonsmokers</td>
<td>0.789±0.056</td>
<td>( \alpha_s ) nonsmokers = 0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( \alpha_r ) smokers</td>
<td>0.472±0.186</td>
<td>( \alpha_r ) smokers = 0</td>
<td>0.0434</td>
</tr>
<tr>
<td>( \alpha_r ) nonsmokers</td>
<td>0.818±0.043</td>
<td>( \alpha_r ) nonsmokers = 0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( \rho_6 )</td>
<td>0.798±0.131</td>
<td>( \rho_6 ) = 0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>( \rho_6 )</td>
<td>0.798±0.131</td>
<td>( \rho_6 ) = 1*</td>
<td>0.0906</td>
</tr>
</tbody>
</table>

*Hypothseis directly relevant to genotype-by-smoking interaction.

**Discussion**

TAS is a complex trait reflecting homeostasis of redox metabolism, which is highly relevant to nearly all pathological processes, including aging, neurodegenerative disorders, atherosclerosis, carcinogenesis, and inflammatory and immunological reactions. It is affected by the relative contributions of each antioxidant species and the stress of oxidative free radicals. The production and the consumption (by free radicals) of each specific antioxidant will affect the total antioxidant capacity. Although many environmental factors can affect the redox homeostasis, for the first time, the present study demonstrates that the TAS, an indicator of redox homeostasis, is under strong genetic control. Amid considerable environmental interferences, a genetic contribution to such a dynamic process at a level as high as 50.9% is particularly significant. Furthermore, on the basis of a dietary recall questionnaire that was designed to capture at least 85% of total macronutrient intake in this population, we found no significant effect on TAS levels for any of the dietary components tested (including percentage of calories as protein, carbohydrate, fat, and alcohol; data not shown). Although much attention has been given to environmental and dietary effects, the present results highlight the importance of investigating the genetic mechanisms responsible for variation in TAS.

There are several published methods for analyzing the total antioxidant capacity as reviewed by Alho and Leinonen. Apart from ABTS-based analysis used in the present study, total peroxyl radical–trapping potential is another commonly used method. These assays are based on slightly different chemical reactions and are possibly differentially affected by the various biologically important antioxidants. However, most methods, such as total peroxyl radical–trapping poten-
tial or TAS, measure peroxyl-scavenging capacity, and a number of relevant antioxidants, including sulfhydryl groups (mostly albumin), urate, ascorbate, α-tocopherol, and bilirubin, are important contributors to this capacity. Although the presence of these antioxidants in plasma will not stop macromolecule peroxidation, such as LDL oxidation in plasma, the measure of TAS does represent a continuous spectrum in the function of antioxidants and free radicals in plasma. It describes residual antioxidant capacity after the consumption of free radicals in plasma. For a given level of antioxidants in plasma, increased production of ROS will result in a reduced TAS level. On the other hand, increased antioxidant availability in plasma will enhance TAS levels for a given ROS amount. Thus, TAS is a continuous measure of oxidative stress in plasma that is relevant to oxidation-induced pathological processes. It is further estimated that approximately 25% to 35% of the total antioxidant capacity is provided by uncharacterized antioxidants. However, the relative contribution of each type of antioxidant to overall antioxidant capacity and to each specific ROS is unknown and may vary from subject to subject and from assay to assay. Thus, dissecting genes contributing to TAS could lead us to discover novel antioxidants that are biologically relevant.

It should also be noted that the measurement of neither total antioxidant capacity nor individual antioxidants has produced consistent results in the prediction of various diseases. Each measure of antioxidant status has its own limitations, including that used in the present study. Such limitations have severely restricted the clinical usage of oxidative stress–related markers. One of the limitations common to all methods is that we are measuring circulating plasma or serum antioxidant capacity and that this may not accurately reflect intracellular antioxidants in target areas, such as endothelial cells or vascular smooth muscle cells, where most atherogenic processes occur. It is known that intracellular and extracellular antioxidant pools are not fully exchangeable. For example, whereas Cu/Zn-SOD (SOD1) and Mn-SOD (SOD2) and are intracellular superoxide-scavenging enzymes, extracellular SOD (SOD3) is responsible for extracellular superoxide dismutation. Whereas oxidative stress occurs intracellularly, it also occurs extracellularly, such as in plasma with pathological relevance. The oxidatively modified lipoproteins, such as oxidized LDL in plasma, do cause endothelial dysfunction and, hence, atherogenesis. Therefore, antioxidants in plasma will have critical roles in scavenging these free radicals before they insult the vascular wall. Measurements of total antioxidant capacity in plasma will have significant implications for the pathological development and clinical manifestations of atherosclerotic lesions. Although intracellular and extracellular antioxidant pools are diversified and although separate studies are needed to dissect genetic contributions to intracellular antioxidant capacity, understanding the genetic regulation of extracellular antioxidant capacity will provide important clues to the intracellular antioxidant system. This may lead to the identification of gene variants that regulate intracellular antioxidation and that might be used as markers of the intracellular redox status. Given the potential inaccessibility to intracellular assessment of vascular wall antioxidant capacity, using functional genetic variants as a marker is an attractive alternative measure.

The observed significant age-sex interactions with the TAS are intriguing. There was a significant age-related decline in male subjects, whereas women showed relative age stability, except for a trend of increase from the mid 40– to mid 70–year range. Furthermore, men also had much higher TAS than did women at most age groups. Similar findings were also reported in a healthy Finnish population and a Chinese population. Although we can understand that the age-related decline in the male population represents a reduced antioxidant capacity with aging, it is somewhat difficult to explain why TAS levels are lower in females than in males. It is widely accepted that premenopausal females have a lower risk of atherosclerosis and that estrogens may also have antioxidant activities. Our findings have shown not only that oral contraceptives are associated with lower TAS levels but also that females have lower, rather than higher, total antioxidant capacity. Sex differences in TAS levels appear to be opposite the differences in the risk of coronary artery disease, raising an interesting question of whether females might be more susceptible to oxidative stress–related disorders. This is obviously a complex question with many confounding factors to affect the final outcome. Sex-specific differences in each of the contributing antioxidants could hold the key to the answer. On the other hand, the high TAS level in males could paradoxically reflect a high oxidative stress level in male subjects that has stimulated the compensatory upregulation of antioxidants.

The substantially higher additive genetic effect on residual TAS phenotypic variance in smokers is not expected. Cigarette smoking is a known environmental factor with a rich source of free radicals, which can reduce antioxidant capacity. We would expect cigarette smoking to have a strong effect on TAS, which might disguise rather than enhance genetic contributions. However, because plasma antioxidants are directly stressed by cigarette smoke–derived free radicals, cigarette smoking could have triggered biological antioxidant responses that are largely under genetic control. It could also be possible that cigarette smoking has created a more homogeneous environment so that the genetic contribution becomes more readily detectable by the statistical model. Indeed, the relationships between DNA sequence polymorphisms and some phenotypic changes, such as hemostatic proteins, were strengthened in smokers. Thus, more studies on tobacco-gene interactions are needed to provide mechanistic information for such regulations. A potential limitation of this finding is that it is confounded by sex, which is also a strong effector of TAS (Table 2). Sixty percent of the 317 smokers were men, whereas men represented only 41% of the total study group.

Decreased antioxidant activity has previously been reported in some diabetic populations. The association between decreased TAS levels and the use of antidiabetic medications, although not diabetes itself, in the present study, may indicate a threshold effect to the extent that medication use reflects a subset of diabetics who have the largest cumulative exposure to hyperglycemia. However, 2 other surrogate indicators of diabetes severity (fasting glucose and diabetes duration) were not associated with TAS.
In summary, for the first time, we have documented that the additive effects of genes explain >50% of the phenotypic variance in plasma total antioxidant capacity. There is a significant gene-by-smoking effect, in which additive effects of genes explain as much as 83% of phenotypic variance in TAS levels in smokers, whereas the genetic contributions are significantly reduced to only 49% in nonsmokers. Further identification of the individual genes and, ultimately, the sequence variations responsible may provide insight in understanding the vastly inconsistent results from clinical trials using various antioxidants to prevent or treat "presumed oxidative stress."

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