Blood Pressure, Risk of Ischemic Cerebrovascular and Ischemic Heart Disease, and Longevity in \(\alpha_1\)-Antitrypsin Deficiency

The Copenhagen City Heart Study

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Background—Because elastase in \(\alpha_1\)-antitrypsin deficiency may attack elastin in the arterial wall, we tested whether \(\alpha_1\)-antitrypsin deficiency is associated with reduced blood pressure, risk of ischemic cerebrovascular (ICVD) and ischemic heart disease (IHD), and longevity.

Methods and Results—We genotyped 7963 control subjects from the adult general population of Denmark, 1131 Danish patients with ICVD, and 2221 Danish patients with IHD. Compared with \(MM/MS\) individuals, systolic blood pressure was lower by 15 mm Hg in \(ZZ\) homozygotes \((n=6, P=0.03)\) and 9 mm Hg in \(MZ\) heterozygotes with IHD \((n=39, P=0.02)\). Odds ratios for ICVD and IHD in \(MZ\) versus \(MM/MS\) individuals were 0.70 (0.51 to 0.96) and 0.77 (0.61 to 0.98). Finally, mean ages of \(MZ\) and \(MM/MS\) control subjects were 58 and 56 years \((\text{Mann-Whitney}: P=0.008)\), and relative \(\alpha_1\)-antitrypsin \(MZ\) genotype frequencies increased from 20 to 93 years among control subjects \(\chi^2, P=0.002\).

Conclusions—\(ZZ\) \(\alpha_1\)-antitrypsin deficiency and \(MZ\) intermediate deficiency in the context of IHD are associated with reduced blood pressure, and \(MZ\) is associated with reduced risk of ICVD and IHD. Because \(MZ\) heterozygosity was associated with increased age, \(MZ\) heterozygosity could be a beneficial condition. (Circulation. 2003;107:747-752.)

Key Words: blood pressure ■ ischemia ■ heart diseases ■ stroke ■ genetics

When lung tissue is \(\alpha_1\)-antitrypsin deficient, protection from neutrophil elastase is impaired and elastin is slowly destroyed.\(^1\) Over time, this leads to premature chronic obstructive pulmonary disease.\(^1\) Uninhibited activity of neutrophil elastase could also destroy elastic tissue of the arterial wall. An altered relation between elastin and collagen in the vessel wall caused by uninhibited elastase activity could theoretically alter the distensibility of the vessel wall and thus blood pressure\(^2\) and cardiac load. Because low blood pressure is protective against ischemic cerebrovascular disease (ICVD) and ischemic heart disease (IHD), \(\alpha_1\)-antitrypsin deficiency may be associated with reduced risk of development of these diseases\(^3,4\) and thus potentially with increased longevity.

Severe and intermediate \(\alpha_1\)-antitrypsin deficiency is almost entirely caused by the \(Z\) and \(S\) alleles as opposed to the wild-type \(M\) allele in the \(\alpha_1\)-antitrypsin (\(SERPINA1\)) gene.\(^1\) Compared with levels in \(MM\) individuals, plasma \(\alpha_1\)-antitrypsin concentrations are lower by 84% in \(ZZ\), 49% in \(SZ\), 17% in \(MZ\), 7% in \(SS\), and 3% in \(MS\) individuals.\(^5\)

We tested 3 hypotheses. First, we examined whether \(\alpha_1\)-antitrypsin deficiency is associated with reduced blood pressure. Second, we examined whether \(\alpha_1\)-antitrypsin deficiency is associated with reduced risk of ICVD and IHD. Finally, we examined if \(\alpha_1\)-antitrypsin genotype frequencies changed as a function of age.

Methods

Overview

We recruited control subjects and case patients with ICVD and IHD from the Copenhagen City Heart Study and additional case patients from Copenhagen University Hospital. Blood pressure was examined in the entire Copenhagen City Heart Study cohort. Risk of ICVD and IHD was examined with the use of pooling of Copenhagen City Heart Study case patients with case patients from Copenhagen University Hospital. Genotype frequency as a function of age was examined in Copenhagen City Heart Study control subjects.

These studies were approved by the ethics committee for the City of Copenhagen and Frederiksberg (No. 100.2039/91; 0-0629/4) and Copenhagen County (No. KA93125). More than 99% of all control subjects and case patients were white and of Danish descent.

Control Subjects

The Copenhagen City Heart Study (third examination, 1991 to 1994) includes 20- to 93-year-old women and men stratified into 5-year age
groups and sampled with the goal of obtaining a representative sample of the adult Danish general population. We measured blood pressure and performed genotyping in 7963 control subjects without ICVD and IHD.

Case Patients From the Copenhagen City Heart Study
Information on ICVD and IHD was collected and verified up until 1997 by reviewing all hospital admissions and diagnoses entered in the Danish National Hospital Discharge Register, all causes of deaths entered in the Danish National Register of Deaths, and medical records from hospitals and general practitioners.

ICVD in case patients was determined on the basis of sudden onset of focal neurological symptoms (WHO International Classification of Diseases, 8th edition: No. 432-435): Ischemic stroke was focal neurological symptoms lasting ≥24 hours, transient ischemic attack was focal neurological symptoms lasting ≤24 hours, and amaurosis fugax was transient monocular blindness. Patients with cerebral hemorrhage on CT scans were excluded. Of 380 patients with stroke, 202 had definite ischemic stroke, 53 had intracerebral hemorrhage (excluded), and 125 had strokes that could not be classified because of lack of a CT scan. However, given the ratio of cases of intracerebral hemorrhage to all cases with a definite diagnosis (53 of 255), 26 (26/255) of the 432 patients with ICVD probably had intracerebral hemorrhage.

IHD in case patients was determined on the basis of previous myocardial infarction or characteristic symptoms of stable angina pectoris based on location, character and duration of pain, and the relation of pain to exercise (WHO No. 410-414).

We measured blood pressure and performed genotyping in 432 and 861 patients with ICVD and IHD from the Copenhagen City Heart Study.

Case Patients From Copenhagen University Hospital
During 1994 to 2001, we recruited 699 patients with ICVD referred to Copenhagen University Hospital, Rigshospitalet, for outpatient ultrasonography of the carotid artery. ICVD was diagnosed on the basis of ischemic stroke, transient ischemic attack, or amaurosis fugax, together with carotid artery stenosis ≥50%. All patients had CT scans performed, and those with cerebral hemorrhage were excluded.

We further identified 1360 patients with IHD referred for coronary angiography at Copenhagen University Hospital during 1991 to 2001. Experienced cardiologists diagnosed IHD on the basis of characteristic symptoms of stable angina pectoris plus at least one of the following: severe stenosis on coronary angiography, myocardial infarction, or a positive result on exercise electrocardiography.

All 699 and 1360 case patients with ICVD and IHD were genotyped.

DNA Analysis
The Z (342Glu→Lys) and S (264Glu→Val) polymorphisms in the α1-antitrypsin (SERPINA1) gene were identified by multiplex polymerase chain reaction (PCR). Genotype frequencies did not differ significantly from those predicted by the Hardy-Weinberg equilibrium (χ²; M:P = 0.86; S:P = 0.12; Z:P = 0.75).

Blood Pressure
Blood pressure was measured by trained technicians, using a London School of Hygiene Sphygmomanometer on the left arm after 5 minutes’ rest with the subject in the sitting position. Pulse pressure was systolic minus diastolic blood pressure. A single measurement of blood pressure is not reliable or reproducible, mainly because of biological and diurnal variation. Importantly, however, individuals of all genotypes had blood pressure measured randomly within the same time intervals (8 AM to 3 PM), and so any differences would affect all groups. Variation in blood pressure levels would therefore theoretically cause this study to underestimate a potential association between blood pressure and α1-antitrypsin deficiency, rather than the opposite. Several technicians were used throughout the study, and were all blinded to clinical details and genotype information.

Other Covariates
Copenhagen City Heart Study participants reported on weekly alcohol consumption, use of medication, physical activity, menopause in women, and employment. All subjects reported whether they were current smokers, ex-smokers, or lifelong nonsmokers; smokers were current smokers. Body mass index (BMI) was weight divided by height² (kg/m²). Cholesterol, triglycerides, and HDL cholesterol were determined enzymatically (CHOD-PAP, GPO-PAP, Boehringer Mannheim). Diabetes mellitus was self-reported disease treated with insulin, oral hypoglycemic agents, or diet.

Statistical Analyses
Because individuals with MM and MS have similar plasma α1-antitrypsin levels, individuals with other genotypes (ZZ, SZ, MZ, SS) were compared with those with MM and MS combined; however, if only compared with MM individuals, the results of this paper were the same.

We used SPSS to run the Student’s t test, Pearson’s χ² test, Mann-Whitney U test, and ANCOVA. Blood pressure was adjusted for age (in deciles), sex, and female hormonal status (premenopausal on oral contraceptives, postmenopausal without estrogen replacement therapy, postmenopausal with estrogen replacement therapy). Interactions between genotype and sex, smoking, IHD, ICVD, and antihypertensive medication in predicting blood pressure were tested in an ANCOVA; these conditions may modulate an effect of α1-antitrypsin deficiency on blood pressure.

Unconditional logistic regression examined the role of genotype in predicting ICVD and IHD. Linear-by-linear χ² test compared relative α1-antitrypsin genotype frequencies by 10-year age groups.

Results
Characteristics and relative α1-antitrypsin genotype frequencies of participants are shown in Table 1. It is noted that age and sex differed between control subjects and ICVD and IHD.
Blood pressure was adjusted for age, sex, and in women for oral contraceptives, menopause, and estrogen replacement therapy; if this analysis excluded individuals on antihypertensive medication (n=1117), the results were similar to those presented. N° of individuals for each covariate varies slightly according to availability of data.

<table>
<thead>
<tr>
<th>Table 2. Difference in Blood Pressure in (\alpha)-Antitrypsin MZ Versus MM/MS Individuals From the Copenhagen City Heart Study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>No. of Individuals</strong></td>
</tr>
<tr>
<td>All</td>
</tr>
<tr>
<td>Without IHD</td>
</tr>
<tr>
<td>With IHD</td>
</tr>
<tr>
<td>No antihypertensive medication</td>
</tr>
<tr>
<td>Antihypertensive medication</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>Nonsmokers</td>
</tr>
<tr>
<td>Smokers</td>
</tr>
</tbody>
</table>

Blood pressure was adjusted by ANCOVA for age, sex, smoking status, physical activity, cholesterol, body mass index, diabetes mellitus, alcohol consumption, and in women for oral contraceptives, menopause, and estrogen replacement therapy; the models stratified by sex and smoking status did not include sex and smoking adjustment, respectively. \(^*\)Statistic. If these analyses excluded individuals taking antihypertensive medication, the results were similar to those presented. “Smokers” were current smokers. Total No. of individuals for each covariate varies slightly according to availability of data.
When this analysis was stratified by antihypertensive medication and smoking, reduced odds ratios for IHD was statistically significant among nonsmokers and among individuals without antihypertensive medication.

Genotype Frequency as a Function of Age

Mean age in MZ control subjects was higher than in MM/MS control subjects (58 versus 56 years; Mann-Whitney, \( P = 0.008 \)); other genotypes did not differ in age from MM/MS control subjects (all probability values \( P \geq 0.50 \)). Age did not differ between MS and MM control subjects (\( P = 0.85 \)).

Frequency of MZ versus MM/MS individuals in the Copenhagen City Heart Study control subjects increased as a function of age (Figure 3). Frequencies of other genotypes did not change as a function of age (all probability values \( P \geq 0.43 \)). Frequency of MS versus MM control subjects did not differ with increasing age (\( P = 0.67 \)).

Discussion

In the present study, we demonstrate that ZZ homozygotes and MZ heterozygotes (in the context of IHD) have lower blood pressure than MM/MS individuals. Furthermore, MZ heterozygosity is associated with reduced risk of ICVD and IHD. Finally, as MZ heterozygosity was also associated with increased age, MZ heterozygosity could be a beneficial condition.

\( \alpha_1 \)-Antitrypsin is the main proteinase inhibitor in human plasma and has previously been suggested as a guardian of vascular tissue. It is bound to the surface of endothelial cells and may diffuse into the arterial wall from the circulation or be produced locally in the arterial wall. The \( \alpha_1 \)-antitrypsin concentration is relatively high in human aortic atherosclerotic lesions. In agreement with this as well as our findings, earlier reports have also observed reduced blood pressure or reduced stiffness in the abdominal aorta of individuals with \( \alpha_1 \)-antitrypsin deficiency, but earlier studies are few and the results are conflicting. Boomsma et al found lower systolic blood pressure in men but not in women with homozygous \( \alpha_1 \)-antitrypsin deficiency. Thus, there are indications that \( \alpha_1 \)-antitrypsin deficiency might have stronger or earlier effects in men than in women. Our results on reduced blood pressure in MZ versus MM/MS individuals in men but not in women support this hypothesis.

Mechanistically, it seems plausible that MZ intermediate \( \alpha_1 \)-antitrypsin deficiency is associated with reduced blood pressure among individuals with IHD but not among those without. This is because individuals with IHD have severe atherosclerosis, a chronic inflammatory disorder. Lung in-

### TABLE 3. Characteristics of ZZ Homozygotes Identified in the Population at Large

<table>
<thead>
<tr>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>SBP/DBP, mm Hg</th>
<th>Smoking</th>
<th>Employment</th>
<th>Medical Treatment</th>
<th>Alcohol Consumption, g/wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Male</td>
<td>44</td>
<td>27</td>
<td>125/95</td>
<td>Current</td>
<td>Office worker</td>
<td>...</td>
</tr>
<tr>
<td>2. Female</td>
<td>44</td>
<td>23</td>
<td>90/60</td>
<td>Current</td>
<td>Skilled worker</td>
<td>...</td>
</tr>
<tr>
<td>3. Male</td>
<td>49</td>
<td>29</td>
<td>140/105</td>
<td>Nonsmoker</td>
<td>Office worker</td>
<td>...</td>
</tr>
<tr>
<td>4. Female</td>
<td>61</td>
<td>29</td>
<td>140/90</td>
<td>Nonsmoker</td>
<td>Self-employed</td>
<td>...</td>
</tr>
<tr>
<td>5. Female</td>
<td>72</td>
<td>18</td>
<td>110/70</td>
<td>Ex-smoker</td>
<td>Office worker</td>
<td>Asthma/bronchitis medication</td>
</tr>
<tr>
<td>6. Male</td>
<td>85</td>
<td>24</td>
<td>130/85</td>
<td>Ex-smoker</td>
<td>Office worker</td>
<td>...</td>
</tr>
</tbody>
</table>

None of the 6 ZZ individuals were taking antihypertensive medication, cardiac medication, diuretics, cholesterol-reducing medication, rheumatism medication, sleeping pills, tranquilizers, gastric medication, or analgesics. BMI indicates body mass index; SBP, systolic blood pressure; and DBP, diastolic blood pressure.

*Now retired.
flammmation caused by smoking is well known to advance the onset and severity of chronic obstructive pulmonary disease in individuals with α1-antitrypsin deficiency.1 Thus, destruction of elastic tissue in arteries could be accelerated by inflammation present locally. Atherosclerosis recruits inflammatory cells (including neutrophils)18 and may induce elastolysis within vessels.19 Moreover, oxidative stress and proteolytic cleavage of α1-antitrypsin20,21 may provide further imbalance between tissue-degrading enzymes and functional levels of α1-antitrypsin in the atherosclerotic inflamed arterial wall. Because the α1-antitrypsin response to inflammation is reduced in individuals with α1-antitrypsin deficiency, it seems likely that this situation can precipitate arterial damage and lead to reduced blood pressure in MZ heterozygotes with IHD but not in those without. As men at the same age often have more atherosclerosis than women, this may also explain the sex difference observed.

In many studies, only a minor decrease in measures of elastic recoil have been found in MZ nonsmokers.1,2,22–24 It is only a fraction of MZ smokers who have apparent damage to lung elastin. Since ZZ individuals have not been known to have impaired arterial elastin as a main feature of the disease, and lung elastin appears to be most susceptible, changes to the arterial walls in MZ individuals were not likely to be expected. This is in accordance with what we found in MZ individuals without IHD.

Only 10 SZ individuals were identified in the present study in the general population as compared with 39 MZ individuals with IHD. This may explain that the reduction in systolic blood pressure in MZ individuals with IHD became significant, whereas a reduction of similar magnitude in SZ individuals did not reach statistical significance.

As blood pressure is a major risk factor for both ICVD and IHD, α1-antitrypsin deficiency could (through reduced blood pressure) be associated with reduced risk of ICVD and IHD. α1-Antitrypsin deficiency could also lead to less cleaved fragments of α1-antitrypsin in atherosclerotic plaques26 and thereby reduce atherosclerotic inflammation and risk of IHD. Previous results suggest that severe α1-antitrypsin deficiency may reduce the risk of IHD,4,25,26 a question our study was not able to address because of too few ZZ homozygotes. Nevertheless, we did observe a lower risk of both ICVD and IHD in MZ versus MM/MS individuals. These findings could represent chance observations. On the other hand, several arguments favor that the above observations also could represent real phenomena. First, the finding could seem biologically plausible since MZ heterozygosity is associated with reduced blood pressure, at least in those with IHD. Second, the reduced risk of ICVD and IHD were similar, at an ≈20% to 30% reduction in risk. And third, among those free of either disease, the frequency of MZ heterozygotes increased with age, in accordance with a possible protective effect of MZ genotype against these two common diseases of the elderly. The increased frequency of MZ individuals in the population with age is particularly interesting because this has been identified before.27 An alternative explanation for this finding has been suggested to be an effect of possible MZ resistance to tuberculosis, causing increase selection, before effective tuberculosis medications were widely used.28

In the present study, selection bias was possible if severe lung disease in some MZ or ZZ individuals prevented them from participating in the third examination of the Copenhagen City Heart Study; however, the observed numbers of these genotypes did not differ significantly from those predicted by the Hardy-Weinberg equilibrium. Nevertheless, if such bias exists, we may have underestimated the effect of MZ and ZZ genotypes on blood pressure and risk of ICVD and IHD. It should be pointed out that our results on ZZ are based on small numbers of individuals. If correction for multiple comparisons was performed, the reduction in systolic blood pressure among ZZ and MZ, and the reduction in ICVD and IHD risk among MZ versus MM/MS individuals would not be of significance. If one insists on only looking at results after correction for multiple comparisons, the only positive findings in this study seem to be (1) that pulse pressure was lower in ZZ and MZ individuals with IHD versus MM/MS and (2) that relative frequency of MZ increase with age.

Misclassification of genotypes is unlikely, because our DNA assay included control sites for restriction enzyme digestion and because all subjects with SZ or ZZ genotypes were reanalyzed to confirm the diagnosis. Bias caused by investigators’ knowledge of disease or risk factor status seems unlikely because we genotyped our samples without prior knowledge of disease status or blood pressure measurements. Finally, database entry was scrutinized by two different researchers.

In conclusion, our results support a role for α1-antitrypsin deficiency as a blood pressure–reducing variant. Because α1-antitrypsin deficiency may protect against cardiovascular disease rather than promote it, there is no obvious clinical
relevance in assessing genotypes and levels. Thus, our results may primarily be used to understand mechanisms underlying interindividual variation in blood pressure and risk of ICVD and HDI. Finally, our results may indicate a positive selection factor that has led to a high incidence of the Z allele in the population.

Acknowledgments

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