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RESEARCH ARTICLE

Alpha1-antitrypsin heterozygosity plays a positive role in attainment of longevity

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Abstract Genes involved in cardiovascular diseases (CVD) play an opposite role in human longevity. The α1-antitrypsin (AAT) is a serine-protease inhibitor required for the prevention of proteolytic tissue damage, by neutrophil elastase. The role of AAT in CVD has not been definitively assessed and its effect on longevity has not yet fully been studied. To clarify these points, we have studied the distribution of AAT allele variants in 3 cohorts: 127 young patients affected by acute myocardial infarction (AMI), 255 young controls and 143 centenarians from Sicily. The Z allele frequency was most frequent in centenarians (13.3%), intermediate in healthy young controls (3.1%) and less frequent in AMI patients (1.2%) ($P = 0.0000001$). The heterozygous MZ genotype was significantly over represented in centenarians (38/143) and under represented in AMI patients (3/127) with intermediate values in young controls (16/255) ($P = 0.0000001$). After adjustment for well-recognized AMI risk factors, the MZ genotype still predicted a significant negative risk factor for developing AMI in the Sicilian population. Thus, our data show a positive role of MZ heterozygosity in attainment of successful ageing linked to the positive effects of this genotype versus the cardiovascular ischemic diseases.

Keywords AAT · Serine-protease inhibitor (Pi) · AMI · Longevity · Centenarians

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Introduction

Centenarian offspring, that have an increased likelihood of surviving up to 100 years, show a reduced prevalence of cardiovascular diseases (CVD), and less frequency of cardiovascular risk

factors. So, genes involved in CVD play an opposite role in human longevity (Lio et al. 2004; Balistreri et al. 2004; Terry et al. 2004; Caruso et al. 2004, 2005; Grimaldi et al. 2006).

Alpha-1 antitrypsin (AAT), also referred to as α_1-proteinase inhibitor (Pi), is a 52 kD glycoprotein secreted by hepatocytes and, to a lesser extent, by lung epithelial cells and phagocytes, into the plasma at a concentration of 1.9–3.5 mg/ml. It is proposed to play an important role in protecting vulnerable elastic tissue in the lungs, gut, and vasculature from degradation by neutrophil elastase and its severe deficiency is involved in chronic obstructive pulmonary disease (COPD) (De Meo and Silverman 2004; Lomas and Parfrey 2004; Luisetti and Seersholm 2004). The Pi gene, located on chromosome 14q32.1 (OMIM: 107400), is 12.2 Kb in length with seven exons and six introns. The encoded protein includes 394 amino acids with the active site of the enzyme inhibitor at methionine 358. It inhibits a variety of serine proteinases but its preferred target is neutrophil elastase that is bound to active site and permanently inactivated (De Meo and Silverman 2004; Lomas and Parfrey 2004).

More than 100 genetic variants of AAT have been identified and these are strictly associated with specific AAT plasma levels in a co-dominantly inherited fashion. Current listings of all polymorphisms can be derived by visiting a database found at: http://www.innateimmunity.net (De Meo and Silverman 2004; Luisetti and Seersholm 2004). The most common allele is PiM; MM homozygotes product a normal level of AAT. Other common variants are PiZ and PiS. The PiZ variant, Glu^{342} to Lys, results in a severe protein deficiency that is characterised in the homozygote state by levels of plasma concentrations lower by 84% when compared with levels in MM individuals and in the MZ heterozygote state by intermediate levels lower by 17%. So, Z homozygosity is a proven genetic risk factor for COPD and emphysema. The PiS variant, Glu^{264} to Val, is associated in homozygosity with AAT levels lower by 7% when compared with levels in MM and in MS individuals with AAT levels lower by 3%. This S variant is not associated to an increased chance of the symptomatology by deficiency syndrome. However, PI MZ and PI MS individuals may have AAT serum protein levels that overlap with PI MM individuals (De Meo and Silverman 2004; Lomas and Parfrey 2004; Luisetti and Seersholm 2004; Needham and Stockley 2004; Cox 1995).

So far, the role of AAT in CVD has not been definitively assessed. A Danish study (Dahl et al. 2003) found that ZZ AAT deficiency and MZ intermediate deficiency patients affected by coronary artery disease (CAD) have lower blood pressure than MM/MS patients. Furthermore, MZ heterozygosity was associated with reduced risk of ischemic cerebrovascular disease (ICVD) and CAD. The authors supposed that neutrophil elastase can play a favourable role in CVD because destroying elastic tissue of the arterial wall, it alters the distensibility of the vessel wall and thus blood pressure and cardiac load. Since low blood pressure is protective against ICVD and CAD, AAT deficiency might be associated with reduced risk of development of these diseases through this mechanism (Ooyama and Sakamato 1995; Zureik et al. 2002; Dahl et al. 2003). However, in that year, another report showed seemingly contrasting results (Talmud et al. 2003). In fact, the other authors demonstrated that the progression of atherosclerosis was associated with low AAT levels. They suggested that elastase activity attributable to AAT deficiency accelerates hardening of arterial walls and atherosclerosis (Talmud et al. 2003). However, this analysis was very limited in scope and numbers (several dozens of patients), and mostly dealt with other variants.

To date, the lifespan of MZ carriers has not yet fully been analysed. Although in the Danish study MZ heterozygosity was associated with increased age, centenarians, the best model of successful ageing (Franceschi et al. 2005), were not studied (Dahl et al. 2003). It is conceivable that lifespan of MZ subjects should depend on the susceptibility to COPD, a life-threatening disease. However, the risk of COPD in PiMZ heterozygotes has been analyzed in several studies, without consistent results. Studies performed to date have both supported and refuted an increased risk of COPD in PI MZ individuals, with case control studies typically finding some increased risk for COPD in PI MZ subjects and population based

survey often finding similar pulmonary function levels in PI MZ and PI M subjects (Seersholm et al. 2000; De Meo and Silverman 2004; Seersholm 2004; Hersh et al. 2004).

Comparison of the DNA sequences of healthy young people with the healthy, extremely old population may reveal genes that heavily participate in the determination of long life (Bessenyei et al. 2004). Thus, to clarify the role of AAT in CVD and longevity, we have studied the distribution of AAT allele variants in acute myocardial infarction (AMI) patients, age-matched controls and centenarians.

Materials and methods

We enrolled 127 patients admitted at the Cardiac Unit of Palermo University Hospital (Italy) as they were affected by AMI. To improve the power of our study, we selected genetically loaded cases having early AMI onset (Marenberg et al. 1994). The diagnosis of AMI was based on typical electrocardiography changes and increased serum activities of relevant enzymes and confirmed by echocardiography and coronary angiography. The healthy age matched control group of 255 subjects was recruited amongst students or staff personnel who were checked and judged to be in good health based on their clinical history and on blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C reactive protein, liver function tests, iron, proteins, cholesterol, triglycerides). The second control group consisted of 143 Sicilian centenarians, whose age was verified by archival records at the City Hall and/or Church registries. We paid particular attention to the concordance between reported age and personal chronologies (age of marriage and of military service for men, age of first and last pregnancy for women, age of children, among others). They did not have any cardiac risk factors or major age-related diseases (e.g., CAD, severe cognitive impairment, severe physical impairment, clinically evident cancer or renal insufficiency), although some had decreased auditory and visual acuity. The characteristics of patient and control groups are reported in Table 1. The Sicilian ethnicity of the participants at the study was established by confirming that all four grandparents were born in Sicily; immigration and intermarriage has historically been rare. The University Hospital Ethics Committee approved the project and an informed consent was obtained from each individual.

Blood specimens were collected in tripotassium EDTA sterile tubes, DNA extracted (Miller et al. 1998) and processed for genotyping according to published protocols. Our procedure for mutation analysis was modified from the PCR-mediated site-directed mutagenesis method of Tazelaar et al. (1992) according to Lam et al. 1997. Allele and genotypic frequencies were evaluated by gene count and differences in frequency among the groups. The χ^2 test (2×2 and 3×3 tables, where appropriate) and ODD ratio (OR) with confidence interval (CI) were calculated. The data were tested for the goodness of fit between the observed and expected genotype

Table 1 Characteristics of centenarians, controls and AMI patients (number and percentage)

	Centenarians	Controls	AMI
Men	46 (32.2%)	107 (41.9%)	117 (92.1%)
Women	97 (67.8%)	148 (58.1%)	10 (7.8%)
Age range	>99	20–55	20–46
Positive familial history of coronary heart disease	Not Applicable	13 (5.1%)	70 (55%)
Current Smokers (Current + Former)	Not applicable	51 (20.%) 60 (23.5%)	90 (71%) (108) (85%)
History of type 2 diabetes	9 (6.3%)	0 (0%)	21 (16.5%)
History of obesity	Not applicable	3 (2.4%)	41 (32.3%)
Hypertension	18 (12.6%)	6 (2.3%)	35 (27.6%)
Blood cholesterol levels > 220 mg/dl	0 (0%)	0 (0%)	74 (58.3%)
Triglycerides > 160 mg/dl	0 (0%)	0 (0%)	52 (41%)

None of subjects referred any case of AAT deficiency in his family history

frequencies according to Hardy-Weinberg equilibrium (HWE), by χ^2 test. Due to the different number of women in the groups under study, logistic regression analysis was performed only in men. We performed a multiple logistic regression analysis to test the association of MZ genotype with AMI taking into account smoking habits, family history of CAD and the presence of type 2 diabetes, obesity, hypertension, hypercholesterolemia and hypertriglyceridemia. The OR (with CI) was calculated as exponential of regression coefficient and its standard error.

Results

Table 2 shows the frequency of AAT M, Z, S alleles in the groups under study. The Z allele frequency was most frequent in centenarians (13.3%), intermediate in healthy young controls (3.1%) and less frequent in AMI patients (1.2%) ($P = 0.0000001$). None of the Sicilian subjects was the ZZ, SS homozygotes and SZ heterozygotes. The heterozygous MZ genotype was significantly over represented in centenarians (38/143) and under represented in AMI patients (3/127) with intermediate values in young controls (16/255) (Table 3, $P = 0.0000001$). All the genotypes were in HWE.

By performing a series of 2×2 separate comparisons, high significance for the different frequencies of MZ genotypes and Z alleles was obtained between centenarians and both young controls and AMI patients. The frequency of MZ genotype and Z allele in AMI patients was lower than that observed in young healthy controls, but the datum was not significant. Finally, we performed a logistic regression analysis to test the association of genotypes with AMI after taking into account smoking habits, family history of CAD and the presence of type 2 diabetes, obesity, hypertension, hypercholesterolemia and hypertriglyceridemia. By performing a multiple logistic regression analysis to test the association of MZ genotype with AMI considering the previous risk factors, significant differences in genotype frequency were observed between 117 male AMI patients and 107 male young controls and persisted between 117 male AMI patients and 46 male centenarians (Table 4). These results indicate that the MZ genotype is an independent negative risk factor for developing AMI in the male Sicilian population.

Discussion

The risk of CVD in patients with severe AAT deficiency has not been sufficiently investigated to formulate firm conclusions. There should be a theoretical association between aortic aneurismal disease and AAT deficiency due to uninhibited

Table 2 Frequency of alleles of AAT in 143 Centenarians, 127 AMI patients and 255 controls from Sicily

Alleles	Allele M	Allele Z	Allele S
Centenarians	242 (85%)	38 (13.3%)	5 (1.7%)
AMI Patients	249 (98%)	3 (1.2%)	2 (0.8%)
Young controls	486 (95.3%)	16 (3.1%)	8 (1.6%)

Significance was obtained by χ^2 (3×3 table) as the alleles were distributed unevenly between the three cohorts ($P = 0.0000001$)

Table 3 Prevalence of the heterozygous MZ genotype in 143 Centenarians, 127 AMI patients and 255 controls from Sicily

Genotypes	MM	MZ	MS
Centenarians	100 (70%)	38 (26.5%)	5 (3.5%)
AMI Patients	122 (96%)	3 (2.4%)	2 (1.6%)
Young controls	231 (90.5%)	16 (6.3%)	8 (3.2%)

Significance was obtained by χ^2 (3×3 Table) for the MZ genotype which was distributed unevenly between the three cohorts ($P = 0.0000001$)

Table 4 2 × 2 Comparisons between the different groups with odd ratio (OR) and confidence interval (CI)

Centenarians (N = 143)	Young controls (N = 255)	AMI patients (N = 127)
Centenarians (*MZ genotypes*)	$P < 0.0001$ OR 0.185 (0.098–0.35)	$P < 0.0001$ OR 0.06 (0.02–0.22)
Centenarians (*Z allele*)	$P < 0.0001$ OR 0.21 (0.115–0.39)	$P < 0.0001$ OR 0.078 (0.02–0.25)

The frequency of MZ genotype and Z allele in AMI was lower (but not significantly) than that observed in the young control cohort. Taking into account smoking habits, family history of CAD, and the presence of type 2 diabetes, obesity, hypertension, hypercholesterolemia, and hypertriglyceridemia, significant differences in genotype frequency persisted between male AMI patients (N = 117) and male centenarians (N = 46) ($P < 0.0001$, OR 0.0544 (0.015–0.19)) and were found male AMI patients (N = 117) and male young controls (N = 107) ($P < 0.0001$; OR 0.35 (0.01–0.15) (by multiple logistic regression analysis)

elastase activity on the elastic tissue of arterial walls, but this has not been supported in published data nor has an association been shown with intracranial aneurysms (St Jean et al. 1996; Needham and Stockley 2004). However, elastin is a major component of vessel wall elastic lamina, and degradation of elastic fibbers may be important in the lowering of blood pressure, which is a well-known risk factor for CVD and AMI. Increased elastase activity attributable to AAT deficiency has therefore been claimed to play a favourable role in CVD because destroying elastic tissue of the arterial wall, it alters the distensibility of the vessel wall and so blood pressure and cardiac load (Robert et al. 1998; Dahl et al. 2003). In fact, in a Danish study the PI Z and PIMZ phenotypes have been associated with lower blood pressure in men and PI MZ has been associated with a reduced risk of ICVD and CAD (Dahl et al. 2003). However, in another study opposite results have been reported (Talmud et al. 2003). The causes of the discrepancies are not clear, but the inclusion criteria, the study populations, and the measured endpoint differed substantially among the two studies (Dahl et al. 2003; Talmud et al. 2003).

The aim of this report was to investigate whether the variants of AAT gene play an opposite role in AMI and longevity. To this purpose, all the subjects were screened for M, Z and S alleles which are the more common variants. We found a higher frequency of MZ heterozygosity and Z allele in centenarians respect to controls and AMI patients. In agreement with these results, Z allele was overrepresented in longevous and underrepresented in patients with AMI with intermediate value in young controls. These results are not due to bias in allele frequencies assessment in our population since the frequencies of healthy controls are close to those expected from literature data. In Caucasoids the most common alleles are the M variants with allele frequencies of greater than 0.95, the Z variant occurring with a frequency of 0.01–0.03 (de Serres et al. 2003; Lomas and Parfrey 2004). So, according to Danish data, our results support a protective role for Z allele in CVD. Besides, they show a positive role of MZ heterozygosity in attainment of successful ageing likely linked to the positive effects of this genotype versus the cardiovascular ischemic diseases.

Concerning the meaning of our results, as previously discussed, a better control of blood pressure in AAT deficiency has been proposed (Robert et al. 1998; Dahl et al. 2003). However, it has to point out that the interaction of AAT with elastase results in the suicide cleavage of AAT. During this process, the C-terminal 36 amino acids (C-36) are cleaved off yet remain attached to the complex. This C-36 cleaved peptide is biologically active and increases low-density lipoprotein (LDL) binding and internalization, upregulates the LDL-receptor, induces cytokine and glutathione production. Thus, there is strong suggestive evidence that C-36 participates in inflammatory processes involved in atherogenesis. AAT deficiency could lead to less cleaved fragments of AAT in atherosclerotic plaques and thereby reduce atherosclerotic inflammation and risk of AMI (Dichtl et al. 2000).

Therefore, present results add another piece of evidence to our hypothesis that people genetically predisposed to a reduced cardiovascular risk, have less chance to develop CVD and, therefore, greater chance to live longer. Genetic polymorphisms responsible for a low inflammatory response may result in an increased chance of

long lifespan in an environment with a reduced pathogen burden and improved control of severe infections by antibiotics since such a modern and healthy environment permits a lower grade of survivable atherogenic inflammatory response (Lio et al. 2004; Balistreri et al. 2004; Caruso et al. 2004, 2005; Grimaldi et al. 2006; Licastro et al. 2005; Candore et al. 2006a, b). In any case, all these data point out the strong relationship between genetics, CVD and longevity.

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