# Age-dependent dichotomous effect of superoxide dismutase Ala16Val polymorphism on oxidized LDL levels

George V. Dedoussis<sup>1,3</sup>, Stavroula Kanoni<sup>1</sup>, Demosthenes B. Panagiotakos<sup>1</sup>, Eirini Louizou<sup>1</sup>, Efi Grigoriou<sup>1</sup>, Christina Chrysohoou<sup>2</sup>, Christos Pitsavos<sup>2</sup> and Christodoulos Stefanadis<sup>2</sup>

<sup>1</sup>Department of Dietetics-Nutrition Harokopio University, 70 El.Venizelou Avenue 17671 Athens, Greece <sup>2</sup>First Cardiology Clinic School of Medicine, University of Athens Ippocration Hospital, 114 Vas Sofias Avenue, 11527 Athens, Greece <sup>3</sup>Corresponding author: Tel, 30-210-9549304; Fax, 30-210-9577050; E-mail, dedousi@hua.gr

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Abbreviations: BMI, body mass index; Cu/Zn SOD, copper- and zinc containing superoxide dismutase; GPX, glutathione peroxidase; Mn SOD, manganese superoxide dismutase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SOD, superoxide dismutase

# Abstract

We investigated the association between superoxide dismutase (SOD) Ala16Val polymorphism and the levels of oxidized LDL lipoprotein-C (ox-LDL-C) in two age-different Greek cohorts. Four hundred fifteen middle-aged (n = 147 females: 43.2  $\pm$  13 years, n = 268males: 43.3  $\pm$  14 years) Caucasian Greek subjects consisted the middle aged cohort. One hundred seventy five elderly (n = 88 females: 79.9  $\pm$  4 years; n = 87males: 80.6  $\pm$  4 years) were selected from the elderly cohort. Genotype data were obtained for all of them. Multiple linear regression analysis, stratified by gender and adjusted for age, smoking habits and body mass index as covariates, showed higher ox-LDL-C levels for the middle aged men with the Val/Val genotype, compared to the other allele (Ala/Ala and Ala/Val) carriers (65.9  $\pm$  25.7 vs. 55.7  $\pm$  20.5 mg/dl; standardized  $\beta$  coefficient = 0.192, *P* = 0.012). On the contrary, elderly women with the Val/Val genotype occurred with lower ox-LDL-C levels compared to the Ala/Ala or Ala/Val genotype (74.2  $\pm$  22.1 vs. 86.5  $\pm$ 26.6 mg/dl; standardized  $\beta$  coefficient = -0.269, P = 0.015). The same trend was also recorded in elderly men, however without reaching statistical significance (standardized  $\beta$  coefficient = -0.187, P = 0.077). Moreover, elderly men and women with the Ala/Ala or Ala/Val genotype presented higher triglycerides levels compared to Val/Val (women: 145.2  $\pm$  68.7 vs. 114.3  $\pm$  34.3 mg/dl, P = 0.027; men: 147.8  $\pm$  72.4 vs. 103.7  $\pm$  38.0 mg/dl, P = 0.002). Additionally, middle aged men with the Val/Val genotype had higher HDL-C levels compared to the Ala allele carriers. The results suggest that SOD Ala16Val polymorphism is an age-dependent modulator of ox-LDL-C levels in middle-aged men and elderly women.

**Keywords:** aging; aged, 80 and over; oxidized low density lipoprotein; polymorphism, genetic; super-oxide dismutase

# Introduction

Aging is an inevitable, heterogeneous but inducible process, characterized by a general decline in physiologic function with an increasing morbidity and mortality rate. Specific causes of aging are not completely elucidated, although various lines of evidence implicate random (stochastic) events as a fundamental driving force behind this process. The free radical theory of aging was postulated as early as 1956 by Harman (1956) and it may explain many of the physiologic changes associated with aging. According to this hypothesis, different types of free radicals are formed in aerobic organisms, particularly due to their metabolic activity. The most important free radicals are the reactive oxygen species (ROS), which have been shown to damage a whole spectrum of cellular key components, during the aging process (Gutteridge and Halliwell, 2000). Free radicals and reactive oxygen and nitrogen species (ROS, RNS) are mutagenic compounds, known to cause DNA damage, favor cell transformation, and contribute to the development of a variety of malignant diseases.

Atherosclerosis is one of the major health concerns in developed countries. It is also well-known, that atherosclerosis is closely related to lipoprotein metabolism. In particular, oxidative modification of LDL is involved in the early development of atherosclerotic lesions, through the formation of macrophage-derived foam cells. Subsequently, ROS formation and oxidative stress contribute to the atherogenetic process (Rosenblat *et al.*, 2002). Several studies have demonstrated the presence of lipid peroxides and byproducts of lipid peroxidation in LDL-C particles and in arterial macrophages in atherosclerotic lesions (Mertens and Holvoet, 2001; Rosenblat *et al.*, 2002). Similarly, it is assumed that lipid peroxidation, subsequent to free-radical damage, is involved in the aging process (Schwenke, 1998).

Several enzymes with antioxidant activity [superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase] comprise a fundamental system, developed by aerobic organisms, to prevent oxidative damage. SODs are the only enzymes dismounting superoxide radicals. There are numerous mechanisms by which cells defend themselves against oxidants (Kinnula and Crapo, 2004). SODs decompose superoxide radicals into H<sub>2</sub>O<sub>2</sub> and O<sub>2</sub> (Kinnula and Crapo, 2004). Mammalian cells, in particular, contain a manganese superoxide dismutase (Mn SOD/SOD2) localized within the mitochondrial matrix (Weisiger and Fridovich, 1973), a copper- and zinc-containing superoxide dismutase (Cu/Zn SOD/SOD1) localized predominantly in cytoplasmic and nuclear compartments (McCord and Fridovich, 1969), and a copper- and zinc containing SOD predominantly found in extracellular compartments (EC SOD/SOD3) (Marklund, 1982). MnSOD, a homotetramer containing one manganese ion per subunit (Beyer et al., 1991; Wan et al., 1994), is essential for the vitality of mammalian cells. The MnSOD gene is located on chromosome 6q25 (Wan et al., 1994). Total knockout of the MnSOD gene is perinatally lethal, leading to neurological manifestations and cardiotoxicity (Tsan, 2001; Van Remmen et al., 2001). Heterozygous mice with decreased MnSOD activity present increased mitochondrial oxidative damage (Williams et al., 1998). A polymorphic variant of MnSOD, with possible enhancing effect on the risk of cancer development, has been described. In this variant, a substitution of T to C base results in the replacement of alanine (Ala) with valine (Val) at position 16 of the mitochondrial targeting sequence (16th amino acid from the beginning of the signal sequence or 9th amino acid from the first amino acid of the mature protein) (Rosenblum et al., 1996). This amino acid replacement has been suggested to change the mitochondrial targeting of the enzyme. It has recently been shown, that the Ala MnSOD/mitochondrial targeting sequence allows efficient targeting of MnSOD to the mitochondria, whereas the Val variant leads to a decreased formation of active MnSOD in the mitochondrial matrix (Sutton et al., 2003).

To examine the influence of the SOD Ala16Val polymorphism on the levels of oxidized low-density

lipoprotein (ox-LDL) during the ageing process, we studied two age different Greek cohorts.

# **Materials and Methods**

The gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey *et al.*, 2001).

## Healthy adults and very old people

Middle aged cohort: The middle aged cohort included 415 middle-aged subjects. This group was part of the "ATTICA" study (Pitsavos et al., 2003), which is a health and nutrition survey, carried out in the province of Attica (including 78% urban and 22% rural areas), where Athens, is a major metropolis. The sampling was performed in the working or other public places; it was random, multistage and based on the age-sex distribution of the province of Attica provided by the National Statistical Service (census of 2001). The participation rate was 68%. From the sampling we excluded 10% of men and 7% of women with history of chronic disease (renal failure, liver and cardiovascular disease and chronic obstructive pulmonary disease), as well as individuals (< 2%) who were under current or chronic use of certain drugs that influence antioxidant status, like methotrexate, trimethoprin, cholestyramine and cyclosporine. Also, all people living in institutions were excluded from the study. All participants were interviewed by trained personnel (cardiologists, dieticians and nurses), using a standard questionnaire.

Elderly cohort: The elderly subjects included in the present study, were part of a large elderly cohort called GHRAS (Greek Health Randomized Aging Study), consisting of 800 healthy men and women older than 65 years of age (unpublished data). The elderly cohort selected for the present study is a sample of 175 subjects older than 75 years of age. The selection criteria was the equal representation all the Ala16Val SOD genotypes in both genders of elderly subjects. Furthermore, the oldest participants had to be free of medication such as antilipidemics, anticoagulants, steroids, diuretics, anticonvulsants, anti-depressive drugs, antibiotics, antimetabolites, non-steroid anti-inflammatory drugs and micronutrient supplementation. Subjects were excluded if they had autoimmune, neurodegenerative, cardiovascular, kidney or liver disease, diabetes, infections, cancer, chronic inflammatory bowel disease or acrodermatitis enteropathica, sickle cell anaemia, chronic skin ulcerations and endocrine disorders. Medical history was recorded by the family doctor or

through a medical examination when possible.

#### Genotyping

Genomic DNA was extracted from whole blood leukocytes with a DNA extraction kit (Nucleospin Blood kit, Macherey-Nagel, Düren, Germany). Ala16Val SOD genotype was determined, as previously described (Masry *et al.*, 2005), for 415 subjects of the middle aged cohort and 212 of the elderly cohort.

#### **Biochemical analysis**

Blood samples were collected after 12 h fasting. The biochemical analysis included total cholesterol, HDL-C, triglycerides and glucose measurements. LDL-C was calculated using the Friedewald equation. Ox-LDL-C was measured in plasma samples using a commercial ELISA kit (Mercodia AB, Uppsala, Sweden), following the manufacturer's instructions.

# Anthropometric, clinical, demographic and lifestyle characteristics

The anthropometric measurements included weight and height and were obtained using standardized techniques and equipment. Body mass index (BMI) was calculated as weight (kg) / height (m) squared. Arterial blood pressure was measured 3 times on the right arm with a sphygmomanometer and with the subject in a sitting position for 30 min. Demographic (age and gender) and lifestyle characteristics (smoking habits) of the middle-aged and the elderly participants were recorded using standardised questionnaires. Current smokers were defined as those who smoked at least one cigarette per day.

#### Statistical analysis

Continuous variables are presented as mean  $\pm$  standard deviation, while categorical variables are presented as absolute and relative frequencies. Pearson's correlation coefficient was used in order to measure associations between normally distributed continuous variables. Contingency tables with the calculation of chi-squared test (with Yates correction) evaluated the associations between categorical variables. However, due to the small number of observations in some cases, Fisher's exact test, with the calculation of exact *P*-values, was applied to evaluate the association between the investigated polymorphism and group of study. The application of Student's *t*-test evaluated the

associations between categorical and normally distributed continuous variables. The distribution of the SOD polymorphism in both populations was compared with the expected frequency through the Hardy-Weinberg equilibrium (HWE). Testing deviations from the HWE was performed by Pearson's chi-squared test, using the observed genotype frequencies obtained from the data and the expected genotype frequencies obtained using the HWE. The association of genotypes with ox-LDL-C concentrations were tested by using multiple linear regression models, after taking into account the effects of several potential confounders. All reported P values are based on two- sided tests and were compared with a significance level of 5%. Statistical analysis was performed with SPSS edition 13.0.

## Results

We evaluated the prevalence of the SOD Ala16Val polymorphism and its effect on ox-LDL-C levels in two age different Greek cohorts, a middle aged one (*n* = 147 females:  $43.2 \pm 13$  years, *n* = 268 males: 43.3  $\pm$  14 years) and an oldest old cohort (females: 79.9  $\pm$  4 years; males: 80.6  $\pm$  4 years). The allele frequency observed in both populations of the current study was 0.54 for Ala and 0.46 for Val. Genotypes frequency was in HWE. Table 1 presents anthropometrical, clinical and biochemical variables of the two cohorts, according to the SOD Ala16Val genotype. Middle aged men with the Val/Val genotype had higher ox-LDL-C and HDL-C levels compared to the Ala carriers (Ala/Ala or Ala/Val) (65.9  $\pm$  25.7 vs. 55.7  $\pm$  20.5 mg/dl, P = 0.011 for the ox-LDL-C; 47.4  $\pm$  12.7 vs. 43.5  $\pm$ 10, P = 0.020 for the HDL- C). Furthermore, middle aged men with the Val/Val genotype had lower total cholesterol/ox-LDL ratio compared to the Ala carriers (Table 1). On the contrary, elderly women with the Ala/Ala or Ala/Val genotype presented higher ox-LDL-C levels and lower total cholesterol/ ox-LDL ratio compared to the Val/Val genotype  $(86.5 \pm 26.6 \text{ vs. } 74.2 \pm 22.1 \text{ mg/dl}, P = 0.042 \text{ for}$ the ox-LDL; 2.9  $\pm$  0.7 vs. 3.2  $\pm$  0.8, *P* = 0.029 for the total cholesterol/ox-LDL ratio). Both elderly men and women with the Ala/Ala or Ala/Val genotype occurred with higher triglycerides levels compared to the Val/Val group (147.8  $\pm$  72.4 vs. 103.7  $\pm$  38.0 mg/dl, P = 0.002 and 145.2  $\pm$  68.7 vs.  $114.3 \pm 34.3$  mg/dl, *P* = 0.027, for men and women respectively). Multiple linear regression analysis (Table 2), stratified by gender and adjusted for age, body mass index and smoking habits as covariates, confirmed the dichotomous age effect of the

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Table 1.	Characteristics	of	the	participants	by	genotype	for	the	Ala16Val	mutation	of	the	SOD	gene.
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Middle aged schort		Women		Men				
Middle aged conort	Ala/Ala, Ala/Val	Val/Val	Р	Ala/Ala, Ala/Val	Val/Val	Р		
n	124	23		216	52			
Age (y)	$38.9\pm11.5$	$\textbf{38.7} \pm \textbf{11.3}$	0.924	$\textbf{45.7} \pm \textbf{9.8}$	$46.0\pm9.2$	0.834		
BMI (kg/m <sup>2</sup> )	$\textbf{23.8} \pm \textbf{4.4}$	$25.7~\pm~5.7$	0.075	$\textbf{27.8} \pm \textbf{4.1}$	$\textbf{27.3} \pm \textbf{3.4}$	0.409		
Systolic Blood Pressure (mmHg)	113.6 $\pm$ 16.4	113.3 $\pm$ 15.7	0.932	127.9 $\pm$ 17.3	128.7 $\pm$ 19.0	0.772		
Diastolic Blood Pressure (mmHg)	76.7 $\pm$ 14.0	78.0 $\pm$ 13.6	0.661	84.7 $\pm$ 15.8	$\textbf{97.0} \pm \textbf{94.8}$	0.365		
Total Cholesterol (mg/dl)	179.6 $\pm$ 39.3	184.1 $\pm$ 37.5	0.612	197.4 $\pm$ 33.7	195.7 $\pm$ 36.6	0.749		
HDL-C (mg/dl)	52.6 $\pm$ 10.9	51.3 $\pm$ 12.5	0.619	43.5 $\pm$ 10.0	47.4 $\pm$ 12.7	0.020		
LDL-C (mg/dl)	110.4 $\pm$ 35.3	108.6 $\pm$ 35.8	0.828	127.5 $\pm$ 31.4	122.6 $\pm$ 34.1	0.329		
Triglycerides (mg/dl)	$84.6\pm44.9$	120.9 $\pm$ 111.0	0.136	130.2 $\pm$ 74.3	130.4 $\pm$ 61.8	0.986		
Oxidized LDL-C (U/L)	$\textbf{61.3} \pm \textbf{32.3}$	$\textbf{65.9} \pm \textbf{29.0}$	0.532	55.7 $\pm$ 20.5	65.9 $\pm$ 25.7	0.011		
Total Cholesterol/Oxidized LDL-C	$\textbf{3.7}~\pm~\textbf{2.2}$	$\textbf{3.3}~\pm~\textbf{1.3}$	0.207	$4.0\pm1.4$	$\textbf{3.4}~\pm~\textbf{1.5}$	0.029		
Glucose (mg/dl)	87.2 $\pm$ 11.3	101.2 $\pm$ 57.8	0.260	$97.0\pm24.1$	104.3 $\pm$ 35.9	0.169		
Current smokers (%)	42.0	39.0	0.800	48	46	0.790		
		Women		Men				
Elderly conort	Ala/Ala, Ala/Val	Val/Val	Р	Ala/Ala, Ala/Val	Val/Val	Р		
n	62	26		65	22			
Age (y)	$80.5\pm4.4$	78.3 $\pm$ 3.5	0.023	$80.7\pm4.3$	$80.3\pm5.3$	0.750		
BMI (kg/m <sup>2</sup> )	$\textbf{29.3} \pm \textbf{4.3}$	$\textbf{30.1} \pm \textbf{4.6}$	0.454	$\textbf{28.1} \pm \textbf{3.6}$	$\textbf{28.7} \pm \textbf{4.0}$	0.553		
Systolic Blood Pressure (mmHg)	147.0 $\pm$ 21.4	139.2 $\pm$ 20.9	0.147	150.6 $\pm$ 17.1	143.6 $\pm$ 18.0	0.128		
Diastolic Blood Pressure (mmHg)	76.7 $\pm$ 10.8	79.1 $\pm$ 12.0	0.384	79.1 $\pm$ 10.7	77.5 $\pm$ 12.5	0.585		
Total Cholesterol (mg/dl)	$\textbf{235.1} \pm \textbf{34.5}$	$\textbf{226.5} \pm \textbf{40.8}$	0.321	$\textbf{227.4} \pm \textbf{36.4}$	$\textbf{210.8} \pm \textbf{45.8}$	0.087		
HDL-C (mg/dl)	55.5 $\pm$ 12.4	60.1 $\pm$ 12.3	0.112	52.3 $\pm$ 10.7	51.1 $\pm$ 9.0	0.646		
LDL-C (mg/dl)	150.6 $\pm$ 32.5	143.5 $\pm$ 39.2	0.389	145.5 $\pm$ 32.4	138.9 $\pm$ 38.9	0.434		
Triglycerides (mg/dl)	145.2 $\pm$ 68.7	114.3 $\pm$ 34.3	0.027	147.8 $\pm$ 72.4	103.7 $\pm$ 38.0	0.002		
Oxidized LDL-C (U/L)	$86.5\pm26.6$	74.2 $\pm$ 22.1	0.042	$81.3\pm21.8$	73.1 $\pm$ 19.1	0.119		
Total Cholesterol/Oxidized LDL-C	$\textbf{2.9}\pm\textbf{0.7}$	$\textbf{3.2}\pm\textbf{0.8}$	0.029	$\textbf{2.9} \pm \textbf{0.7}$	$3.0\pm0.5$	0.817		
Glucose (mg/dl)	104.2 $\pm$ 29.0	95.0 $\pm$ 12.9	0.127	110.2 $\pm$ 33.6	102.0 $\pm$ 20.7	0.288		
Current smokers (%)	8.1	11.5	0.689	15.4	4.5	0.277		

Table 2. Results from multiple linear regression models that evaluated the association between oxidized LDL-C concentrations and SOD genotype stratified by gender\*.

Middle-aged cohort	Women		Men			
Variable	Standardized $\beta$ coefficient	Р	Standardized $\beta$ coefficient	Р		
Val/Val vs. Ala/Ala, Ala/Val	0.071	0.410	0.192	0.012		
Age (y)	-0.045	0.640	0.100	0.195		
BMI (kg/m²)	-0.094	0.341	-0.137	0.076		
Non-smoking vs. current smoking	0.071	0.175	0.016	0.832		
Adjusted R <sup>2</sup>	0.3%	0.361	4.2%	0.025		
Elderly cohort	Women		Men			
Variable	Standardized $\beta$ coefficient	Р	Standardized $\beta$ coefficient	Р		
Val/Val vs. Ala/Ala, Ala/Val	-0.269	0.015	-0.187	0.077		
Age (y)	-0.186	0.093	-0.337	0.003		
BMI (kg/m²)	0.061	0.568	-0.099	0.364		
Non-smoking vs. current smoking	-0.032	0.759	0.092	0.396		
Adjusted R <sup>2</sup>	4.4%	0.102	8.9%	0.020		

\*Significant genotype  $\times$  sex interaction for both cohorts (*P* < 0.05).

Val/Val genotype on ox-LDL-C levels (standardized  $\beta$  coefficient = 0.192, *P* = 0.012 and standardized  $\beta$  coefficient = -0.269, *P* = 0.015, for middle aged men and oldest old women, respectively). Furthermore, a trend for lower ox- LDL-C levels in old men with the Val/Val genotype was also recorded, with a nominal significance (standardized  $\beta$  coefficient = -0.187, *P* = 0.077).

# Discussion

In this study we evaluated the effect of SOD Ala16Val polymorphism on the ox-LDL-C levels in two age-different groups, a middle aged and an oldest old one. The allele frequency recorded in our populations was inversely distributed, comparing to Asians populations (current study: Ala 0.54 and Val 0.46; healthy Chinese: Ala 0.13 and Val 0.87, healthy Korean: Ala 0.12 and Val: 0.88) (Lee *et al.*, 2006; Mak *et al.*, 2006). On the other hand, the recorded prevalence of the Ala16Val polymorphism in the current study is similar to other Caucasian populations, like Turks (Akyol *et al.*, 2004) and Brazilian residents (Gottlieb *et al.*, 2005).

As oxidative stress is considered to play a critical role in senescence-associated changes, it is conceivable that antioxidant enzymes are important anti-aging agents. Surprisingly, the investigated polymorphism was found to have an inverse effect on ox-LDL-C levels in our two age-different populations. In middle age men, the presence of the Val allele, which according to the literature results to a less active SOD enzyme, was positively associated with ox-LDL-C and HDL-C levels. A possible explanation of the higher HDL-cholesterol levels observed in middle aged men with the Val/Val genotype could be a feed-back mechanism in these subjects where the anti-oxidant properties of HDL might compensate the high oxidative stress represented by the elevated ox-LDL levels (Lee et al., 2005). Our finding of increased HDL cholesterol levels in middle-aged subjects with the Val/Val genotype is in accordance with the results of other studies (Kakko et al., 2003; Lee et al., 2006), where Val/Val subjects present higher HDL-C levels compared to the other genotypes, however without statistical significance. Evidence for the existence of a feed-back mechanism among HDL-C levels and antioxidant enzymes has been demonstrated in subjects with low HDL-C levels (Savor-Blat et al., 2007), as well as in studies with Glutathione peroxidase-1 deficient mice (de Haan et al., 2006). On the other hand, it is well documented, that healthy elderly have higher levels of HDL-C than younger subjects (Schaefer *et al.*, 1989), the vast majority of those over 70 years (~90.0%) occurring with HDL-C levels over 40 mg/dl (Nikkila and Heikkinen, 1990). This increment in HDL-C levels in healthy oldest old subjects (survivors), also recorded by our previous study (Kanoni *et al.*, 2006), could have overshadowed the potential impact of the SOD genotype.

The opposite was recorded in elderly women, where the Val allele was associated with decreased ox-LDL-C, as well as triglycerides levels. A positive correlation of triglyceride with ox-LDL levels observed in elderly has been previously shown for middle aged people (Lapointe et al., 2007). It is well established that higher TG levels in plasma enhance production of small, dense LDL particles, which are known to be more susceptible to oxidation (Young et al., 2003). Data from the Multi-Ethnic Study of Atherosclerosis (MESA) suggest that, dyslipidemia (adverse levels of cholesterol, HDL-cholesterol, triglycerides), inflammation markers (elevated fibrinogen), male gender, ethnicity (black) and current smoking, could explained the most variation in ox-LDL-C levels, in multivariate analysis model (Holvoet et al., 2006b). The same investigators provide evidence, that ox-LDL-C is a proinflammatory stimulus, inhibiting the expression of the antioxidant SOD1 (Holvoet et al., 2006a). Results (Mocchegiani, 2007) from the large European elderly study (Zincage) suggest that erythrocyte's SOD activity (eSOD) significantly and progressively increase with age, while women exhibit higher eSOD activity compared to men in all age groups. Previously reported results regarding the activity of red blood cell (RBC) SOD, plasma GPX, and, to a lesser extent, plasma SOD are contradictory. Some investigators claim an increase from the youngest to the oldest age groups, which has been correlated with a parallel increase of free radicals (Mecocci et al., 2000; Cheynier, 2005). However, in a cross- sectional study (Andersen et al., 1998) conducted in 41 Danish centenarians and 52 control subjects, Cu/Zn-SOD activity decreased in the centenarians group. In the area of endogenous antioxidant defenses, excess amount of ROS leads to the depletion of the protective GSH and SOD (Kaliora et al., 2006). Very old people represent a highly selected group of successfully aged people. They have apparently escaped the major age-related diseases and exhibit special immunologic or metabolic features (Andersen et al., 1998; Biesalski, 2002). In several studies involving young and elderly groups, some investigators have detected a positive association between age and increased LDL-C oxidation and decreased ox-LDL-C antibodies (Mertens and

Holvoet, 2001), while others (Nakamura *et al.*, 2006) not. These contradictory results could be attributed to the different population size.

The promoter of MnSOD gene contains binding sites for several transcription factors such as AP-1, AP-2, SP-1, and NF-κB (Harris et al., 1991; Das et al., 1995). In line with this, MnSOD is induced by multiple stress conditions both in vitro and in vivo. MnSOD induction occurs after exposure to cytokines (TNF-α, IFN-γ, IL-1, IL-6) (Ono *et al.*, 1992), cigarette smoke (Gilks et al., 1998), chronic hypoxia (Clerch et al., 1998), asbestos fibers (Janssen et al., 1992), radiation (Guo et al., 2003) and modulators of intracellular redox and/or thiol state (Das et al., 1995). Most of these parameters are age dependent factors and adding smoking as a cofactor in our model, the effect of SOD polymorphism on ox-LDL-C levels remained statistically significant.

Ox-LDL-C appears to be a stimulator of NF-κB and AP-1, which are thought to play a predominant role in endothelial cell activation, resulting in atherogenesis. It has been recently shown (Lin *et al.*, 2007), that overexpression of Cu, Zn-SOD and/or catalase suppress the ox-LDL-induced increase in AP-1 and NF-κB activity, providing information on a mechanism potentially explaining the antiatherogenic and antiproliferative activities of antioxidant enzymes. Overexpression of MnSOD inhibits, *in vitro*, the oxidation of LDL-C by endothelial cells (Fang *et al.*, 1998) and ox-LDL-C is able to induce the expression of MnSOD in macrophages (Kinscherf *et al.*, 1997).

Many SNPs have a significant effect only in the presence of heterozygosity of a second SNP. This type of interaction would not be detected using single-SNP analysis, because SNP genotypes could have a limited effect on a subset of the population, or even antagonistic effects when investigated in combination with a second SNP genotype. These observations are consistent with the hypothesis, that interactions between genetic variants and environmental exposure are the primary causes of phenotypic variability, and not the genetic variants alone (Dedoussis *et al.*, 2007).

An age-dependent effect of a single gene polymorphism was recently shown in a Finish study (Fan *et al.*, 2007). The results suggested that HL C-480T polymorphism is a strong age-dependent risk factor of sudden cardiac death in middle-aged men. We have no evidence of the underlying mechanism, which could explain the inverse effect of the SOD polymorphism on ox-LD-C levels in the oldest old women. If the decrease in SOD activity, as previously reported, is more evident in the SOD Ala carriers, then this could partially explain the

higher ox-LDL-C levels in these subjects. In line with our results and our hypothesis, a recently published study (Taufer et al., 2005), demonstrated that in elderly, the Ala/Ala genotype was associated with increased risk for cancer, immunosenescence profile and DNA damage. The proposed hypothesis for these results by the investigators is based on the free radical theory of aging, concisely suggesting that Ala carriers effectively produce SOD and thus presenting strong innate immunity in their middle-age life, but increased immunosenescence in their latter life. An age related decline in gene expression was recently (Ling et al., 2004) reported for a common Gly482Ser polymorphism in the transcriptional co-activator PGC-1 $\alpha$  in muscle biopsies. Insulin resistance in skeletal muscle increases with age, and since skeletal muscle is a major site of insulin stimulated glucose disposal, aging is a risk factor for type 2 diabetes (Petersen et al., 2003).

The limitations of our study include the crosssectional design, the absence of multiple antioxidant compounds measurements and the evaluation of other genetic markers related to antioxidant enzymes.

Longitudinal studies in both of our populations, as well as the study of other genes known to affect the levels of ox-LDL-C, will add new evidence on the age specific variation in ox-LDL-C levels and the predisposing genetic markers. To our knowledge this is the first report of a dual effect of a polymorphism on ox-LDL-C levels depending on age.

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