

Paraoxonase Activity and Genotype Predispose to Successful Aging

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The paraoxonase 1 codon 192 R allele has been previously reported to have a role in successful aging. The relationship between PON1 genotypes, enzymatic activity, and mass concentration was evaluated in a group of 229 participants from 22 to 104 years of age, focusing our attention on nonagenarian/centenarian participants. We found a genetic control for paraoxonase activity that is maintained throughout life, also in the nonagenarians/centenarians. This activity decreases significantly during aging and shows different mean values among R and M carriers, where R+ and M– carriers have the significant highest paraoxonase activity. Results from the multinomial regression logistic model show that paraoxonase activity as well as R+ and M– carriers contribute significantly to the explanation of the longevity phenotype. In conclusion, we show that genetic variability at the PON1 locus is related to paraoxonase activity throughout life, and suggest that both parameters affect survival at extreme advanced age.

THE paraoxonase gene family contains at least three members, named PON1, PON2, and PON3, which are located on chromosome 7q21.3-22.1 (1). This enzyme is a high-density lipoprotein (HDL)-bound arylesterase which hydrolyzes lipoperoxides. It is responsible for the protective effects of HDL on LDL (low-density lipoprotein) peroxidation (2,3). Oxidized LDL are pro-inflammatory, pro-atherogenic, and responsible for several phenomena such as foam cell production, cytotoxic effects toward arterial wall cells, and macrophagic cytokine induction. Inter-individual variability of PON1 serum activity has been thought to play an important modulatory role in the risk of developing atherosclerosis (4).

PON1 gene polymorphisms lead to an amino acid substitution (Gln-Arg) at position 192 in the coding region of the gene and to an amino acid substitution (Leu-Met) at position 55. Alleles at codon 192 (Q and R alleles) and codon 55 (L and M alleles) in the PON1 locus have been associated with enzymatic activity and concentration, respectively (5–7). There is wide variation in PON1 protein and activity between individuals even within genotype groups (8). In addition to genetic polymorphisms, PON1 activity can be modified by several factors such as diet, lifestyle, and pathologic states (9). Consistently, a large number of studies suggested an association between major cardiovascular diseases and the Arg 192 (commonly indicated as allele R) variant (10). Also the Leu 55 variant (currently named as allele L) has been associated with cardiovascular disease susceptibility (11). It has also been suggested that the codon 192 R allele does not exert any

significant role on CHD (coronary heart disease) risk in an Italian population (12). In contrast, data indicated that the PON1 genotype affects the responsiveness of vasculature to triglycerides (13). It is likely to be the phenotype and not simply the genotype that is important in the interaction of PON1 with CHD (14,15). The relatively small number of studies taking into consideration PON1 concentration and/or activity data has suggested that PON1 levels are reduced in CHD, and that this effect is independent of PON1 genotype (16). Until now, few studies have investigated the possible role of PON1 variability and survival at extreme advanced age. Our previous papers reported an increase in the frequencies of R allele and R+ carriers both in Irish octo/nonagenarians and Italian centenarians in comparison with their younger controls (17,18). We have supposed that the R allele could decrease mortality in carriers and that centenarians could have a genetically determined paraoxonase activity advantageous for longevity (19). At present, the role of PON1 genotypes and phenotypes in aged people free of disease has not been properly investigated.

In this study we wanted to better explore the role of PON1 during aging, especially focusing our attention on nonagenarians/centenarians, individuals who have reached the extreme limits of human life span. A sample of 229 participants from 22 to 104 years of age was divided into three different subgroups (young, elderly, and nonagenarians/centenarians as reference group) and PON1 genotypes, paraoxonase activity, arylesterase activity, and paraoxonase mass concentration were evaluated to define the role of these

variables in the chance for young and elderly people to become extremely old.

METHODS

Participants

A sample of 77 young participants ranging from 22 to 65 years of age, 56 elderly participants ranging from 66 to 89 years of age, and 96 nonagenarian/centenarian participants were enrolled. All the participants were genetically unrelated, recruited in Central Italy, and appeared healthy at the time of blood collection, as assessed by clinical examination and recent clinical history (20). In particular, centenarians belonged to category A and B of our previous classification (21); those in category C were excluded from this study. Inclusion criteria for category A were the follows: absence of physical disabilities (Activities of Daily Living [ADL] = A, B, or C; Instrumental Activities of Daily Living [IADL] > 4), absence of severe cognitive impairment (Mini Mental State Examination [MMSE] > 20), absence of clinically evidence of cancer, absence of severe renal failure, absence of severe anemia, and absence of severe liver disease. Inclusion criteria for category C were as follows: presence of severe cognitive impairment (MMSE < 12), presence of severe physical impairment (ADL = F-G; IADL = 0), presence of overt cancer, presence of anemia, presence of severe liver diseases, and presence of renal insufficiency. Category B included the centenarians whose health conditions were intermediate between those of categories A and C. All the volunteers were informed about the goal of the study and gave informed, written consent.

PON1 192 and 55 Genotyping

DNA was extracted from blood lymphocytes by the salting-out method (22). Polymerase chain reaction was performed using primer sequences derived from published data and specific for the amplification of the region surrounding codons 192 and 55. The amplification reactions and methodologies have been previously described (17).

Biochemical Profile of Recruited Participants

Serum concentration of the main parameters of lipometabolic balance (fasting glucose, total cholesterol, HDL cholesterol, and triglyceride levels) was measured using commercially available kits on a Roche/Hitachi 912 (Roche Diagnostics, Basel, Switzerland), according to the manufacturer's specifications, and quality control was within the recommended precision for each test.

Paraoxonase Activity, Arylesterase Activity, Paraoxonase Mass Concentration, and Specific Activity

Paraoxonase activity was measured by adding serum to a buffer containing paraoxon (o,o-diethyl-o-p-nitrophenylphosphate; Sigma-Aldrich, Milan, Italy). The rate of generation of p-nitrophenol was measured at 405 nm with the use of a spectrophotometer (Beckman Du-640; Beckman Coulter, Inc., Fullerton, CA) (23). Arylesterase activity in

serum was measured as above using phenylacetate as substrate and setting the spectrophotometer at 270 nm (24).

Serum concentration of paraoxonase was assayed by a competitive enzyme-linked immunoassay (25). Specific activity was determined as U/ μ g with paraoxon as substrate.

Statistical Analysis

Data were analyzed with the SPSS/Win program (version 11.5; SPSS Inc., Chicago, IL). Triglycerides and paraoxonase activity were natural log transformed before statistical analyses to achieve a normal distribution. The corresponding results are shown as geometric means \times/\div antilog standard deviation (SD). Differences among the three age groups (young, elderly, and nonagenarians/centenarians) were initially assessed by univariate analysis using the analysis of variance (ANOVA) test for continuous variables and the χ^2 test for categorical variables. The distributions of the PON1 192 and PON1 55 genotypes and allele frequencies in the two groups were compared by χ^2 test. Hardy-Weinberg equilibrium was checked by Monte Carlo Markov Chain (26).

Multinomial Logistic Regression Model

Because age is defined as a three-level categorical outcome, the multinomial logistic regression was performed to evaluate multivariate relationships between the classified age and a set of covariates (carrier192, carrier55, sex, arylesterase activity, paraoxonase mass concentration, paraoxonase activity). Arylesterase activity, paraoxonase mass concentration, and paraoxonase activity were continuous covariates, and they showed the relative strength of association with the outcome in the regression model. After the deletion of two cases with missing values, data from 227 people were available for the analysis and the multinomial logistic regression generated two logit functions for assessing prediction of people in one of three categories of outcome. Odds ratios (ORs) were estimated for the young and elderly groups with respect to the nonagenarian/centenarian group (reference group) (27). Bonferroni's correction was used to compensate for inflated type I error rate with six predictors. The corrected value for $\alpha = 0.008$ was obtained by dividing $\alpha = 0.05$ by the number of predictors ($n = 6$).

RESULTS

Table 1 shows the demographic, biochemical, and clinical characteristics of the participants divided by age groups. In the nonagenarian/centenarian group, there was a significant overrepresentation of females with respect to the other two groups, as expected from the higher survival of females in the extreme decades of life in Italy as well as in other countries (21,28). Body mass index, paraoxonase activity, arylesterase activity, and paraoxonase specific activity were significantly lower in nonagenarians/centenarians compared to elderly and young individuals. Body mass index and arylesterase activity were also different between elderly and young people. Paraoxonase mass concentration followed a different pattern as it was similar in young people and nonagenarians/centenarians, showing a significant decrease in elderly persons. HDL cholesterol and triglyceride levels were similar in elderly people and in nonagenarians/centenarians, but were higher in these two groups than in the

Table 1. General Characteristics of the Studied Participants

Characteristic	Young (22–65 y) N = 77	Elderly (66–89 y) N = 56	Nonagenarians/Centenarians (≥90 y) N = 96
Age, y	43.8 ± 12.9	75.5 ± 5.6	98.4 ± 5.6
Sex (M %) (M/F)	51.9 (40/37)*	46.4 (26/30) [†]	15.6 (15/81)
BMI, kg/m ²	24.4 ± 1.3* [‡]	26.6 ± 4.1 [†]	23.1 ± 3.9
Fasting glucose, mmol/L	4.96 ± 0.33	5.44 ± 1.17 [†]	4.93 ± 1.65
Total cholesterol, mmol/L	5.09 ± 1.04	5.91 ± 0.78 [†]	5.06 ± 1.13
Triglycerides, mmol/L	0.82 ×/÷ 0.02*	1.15 ×/÷ 0.02	1.09 ×/÷ 0.02
HDL cholesterol, mmol/L	1.29 ± 0.30*	1.43 ± 0.40	1.47 ± 0.43
PON activity, U/ml	292.95 ×/÷ 1.65*	297.14 ×/÷ 1.65 [†]	198.76 ×/÷ 1.82
Arylesterase activity, U/ml	87.5 ± 39.2* [‡]	56.4 ± 16.5 [†]	45.2 ± 15.7
PON mass concentration, µg/ml	103.2 ± 24.9	93.4 ± 20.0 [†]	104.4 ± 28.2
PON specific activity, U/µg	3.4 ± 2.0*	3.8 ± 2.3 [†]	2.4 ± 1.5

Note: Values are arithmetic means ± standard deviation (SD), geometric means ×/÷ antilog SD for skewed data.
 p < .05 for the following comparisons: *Nonagenarians/centenarians vs Young; [†]Nonagenarians/Centenarians vs Elderly; [‡]Elderly vs Young.
 M = male; F = female; BMI = body mass index; HDL = high-density lipoprotein.

group of young people. It was interesting to note that total cholesterol levels were similar in the young people and nonagenarians/centenarians but significantly lower in these two groups than in the elderly people.

All the participants were also genotyped for PON1 192 and 55 polymorphisms. In all the groups, the observed genotypes were in agreement with those expected at Hardy–Weinberg equilibrium (p > .05). The genotypes were grouped as R+ carriers (QR+RR) and R– carriers (QQ) for the codon 192 polymorphism, and as M+ carriers (LM+MM) and M– carriers (LL) for the codon 55 polymorphism. A trend toward a higher frequency of PON1 192 R+ carriers, though not statistically significant, was found in the elderly and nonagenarian/centenarian groups (44.2% for young, 55.4% for elderly, and 53.1% for nonagenarian/centenarian participants), whereas no difference was found between young people and nonagenarians/centenarians for PON1 55 M+ carrier frequencies (61.0% for young, 73.2% for elderly, and 61.5% for nonagenarian/centenarian participants).

In Tables 2 and 3, the mean values of paraoxonase activity, arylesterase activity, paraoxonase mass concentration, para-

oxonase specific activity, and HDL in the three age groups were reported and subdivided according to PON1 R+ and R– carriers (codon 192) and M+ and M– carriers (codon 55).

Paraoxonase activity was significantly decreased with age both in R and M carriers, and the major decrease was present in the nonagenarian/centenarian group. We also found, in the last age group, a genetic control for paraoxonase activity that was maintained throughout life. As shown in Figure 1, the R+ and M– carriers had the highest levels of paraoxonase activity throughout the entire age-range.

Arylesterase activity also decreased significantly in the three age groups for both carriers. The higher decrease was already evident in the elderly group, independently of the polymorphisms. The different levels of arylesterase activity correlate only among M carriers, with M+ participants having the lower levels. Moreover, ANOVA showed an interaction between M carriers and age groups on arylesterase activity (Table 3).

Paraoxonase mass concentration was dependent on age group, with the lowest levels in the elderly group. No differences in paraoxonase mass concentration among R and M

Table 2. Analysis of Variance in the Three Age Groups by PON1 Carrier192

Age	Carrier192	Paraoxonase Activity (U/ml)	Arylesterase Activity (U/ml)	Paraoxonase Mass Concentration (µg/ml)	Paraoxonase Specific Activity (U/µg)	HDL Cholesterol Levels (mmol/L)
20–65 y	R– (n = 43)	194.0 ± 1.4	84.8 ± 39.5	101.6 ± 26.6	2.0 ± 0.4	1.27 ± 0.31
	R+ (n = 34)	493.4 ± 1.3	90.7 ± 39.2	105.1 ± 22.9	5.1 ± 1.9	1.31 ± 0.28
66–89 y	R– (n = 25)	205.7 ± 1.4	52.5 ± 10.8	93.1 ± 19.3	2.4 ± 1.0	1.44 ± 0.38
	R+ (n = 31)	399.7 ± 1.5	59.7 ± 19.4	93.4 ± 20.0	4.9 ± 2.4	1.42 ± 0.43
≥90 y	R– (n = 45)	131.72 ± 1.6	46.5 ± 17.5	103.5 ± 25.8	1.5 ± 0.9	1.56 ± 0.47
	R+ (n = 51)	287.0 ± 1.4	43.8 ± 14.2	103.9 ± 29.2	3.2 ± 1.5	1.39 ± 0.39
Total	R– (n = 113)	168.4 ± 1.6	62.4 ± 32.3	100.5 ± 25.0	1.9 ± 0.9	1.42 ± 0.41
	R+ (n = 116)	367.6 ± 1.5	43.8 ± 14.2	101.5 ± 25.6	4.2 ± 2.1	1.37 ± 0.37
Analysis of variance		F value	F value	F value	F value	F value
Age		38.97*	57.21*	3.43 [†]	20.00*	5.18*
Carrier192		250.44*	0.92	0.19	141.17*	0.94
Age by carrier192		2.23	0.83	0.09	5.15*	1.48

Note: Values are means ± standard deviation.
 *p < .01.
[†]p < .05.
 HDL = high-density lipoprotein.

Table 3. Analysis of Variance in the Three Age Groups by PON1 Carrier55

Age	Carrier55	Paraoxonase Activity (U/ml)	Arylesterase Activity (U/ml)	Paraoxonase Mass Concentration ($\mu\text{g/ml}$)	Paraoxonase Specific Activity (U/ μg)	HDL Cholesterol Levels (mmol/L)
20–65 y	M– (n = 30)	431.2 \pm 1.6	103.6 \pm 41.0	114.6 \pm 21.6	4.4 \pm 2.4	1.34 \pm 0.31
	M+ (n = 47)	228.9 \pm 1.6	77.2 \pm 34.6	103.2 \pm 24.9	2.8 \pm 1.5	1.25 \pm 0.29
66–89 y	M– (n = 15)	421.0 \pm 1.6	58.3 \pm 17.8	89.6 \pm 21.2	5.5 \pm 2.9	1.45 \pm 0.54
	M+ (n = 41)	261.6 \pm 1.6	55.7 \pm 16.2	94.8 \pm 19.6	3.2 \pm 1.7	1.42 \pm 0.35
≥ 90 y	M– (n = 37)	256.8 \pm 1.5	53.7 \pm 14.0	100.2 \pm 27.6	3.0 \pm 1.6	1.48 \pm 0.36
	M+ (n = 59)	169.9 \pm 1.8	45.1 \pm 15.8	105.9 \pm 27.4	2.0 \pm 1.3	1.46 \pm 0.43
Total	M– (n = 82)	339.8 \pm 1.7	72.8 \pm 36.1	103.5 \pm 25.9	4.0 \pm 2.3	1.42 \pm 0.42
	M+ (n = 147)	210.8 \pm 1.7	56.2 \pm 28.2	99.7 \pm 24.9	2.6 \pm 1.5	1.38 \pm 0.38
Analysis of variance		F value	F value	F value	F value	F value
Age		19.76*	64.41*	4.19 [†]	17.26*	4.36 [†]
Carrier55		50.82*	15.43*	0.54	41.80*	0.69
Age by carrier55		1.05	3.21 [†]	5.89*	1.97	0.19

Note: Values are means \pm standard deviation.

* $p < .01$.

[†] $p < .05$.

HDL = high-density lipoprotein.

carriers were found. Moreover, we found that age groups and M carriers interact on paraoxonase mass concentration (Table 3). Mean values of paraoxonase mass showed a different trend in M– carriers with respect to M+ carriers; in the former, we found the lowest levels in the elderly groups, whereas in the latter, we found the highest levels in the nonagenarian/centenarian group.

Mean values of paraoxonase specific activity significantly decreased in the three age groups. In particular, the more evident decrease was present in the nonagenarian/centenarian group. Paraoxonase specific activity showed a genetic control that was maintained throughout life, also in the oldest age group. This specific activity showed different values among R and M carriers, where R+ carriers and M– carriers had the highest values. ANOVA highlighted that age groups and R carriers interact on paraoxonase specific activity (Table 2).

To investigate whether the evaluated parameters were related to the achievement of extreme age, we made use of a multinomial logistic regression model. We decided to evaluate the results obtained from the model by using a conservative approach; therefore, we considered statistically significant $p < .008$ as obtained from the Bonferroni correction (see Methods). The multinomial logistic regression model provided significant likelihood ratio test results for each predictor, except for paraoxonase mass concentration ($-2\text{LR} = 335.71, p = .082$), and verified that the ORs for both comparisons are simultaneously different from zero. There was good model fit on the basis of six predictors using a Pearson criterion ($\chi^2 = 402.87, p = .897$). Comparison of log-likelihood ratios for models with only the intercept and with the predictors showed reliable improvement ($\chi^2 = 330.71, p < .0001$). Individual 95% confidence intervals (95% CI) are presented for each estimated OR value. The results of this analysis are shown in Table 4, in which the OR values (95% CI) for the variables introduced in the model are reported. In the top portion of Table 4, the comparison between the young group versus the nonagenarian/centenarian group (reference group) is reported. The continuous variables (arylesterase and paraoxonase activities) fitted

significantly in the model and they contributed to explain the variability, that is, the longevity. Moreover, as expected in our model, females had an advantage in achieving the extreme limits of human life span (OR, 5.93; 95% CI, 2.33–15.12; $p < .001$). Concerning the relationship between the different carriers and the probability of young people to become centenarians, we found that R+ carrier and M– carrier participants were not significantly associated. Also, paraoxonase mass concentration did not reach statistical significance; thus, its role in longevity is likely negligible.

In the bottom portion of Table 4, the comparison between the elderly group versus the nonagenarian/centenarian group (reference group) is reported. In this case, paraoxonase activity was significantly associated with longevity (OR, 1.01; 95% CI, 1.01–1.02; $p < .001$). Regarding the involvement of R+ and M– carriers and their relationship with longevity, we found that R+ carrier and M– carriers elderly participants have an advantage to become centenarians with ORs of 6.25 (95% CI, 1.71–22.91; $p = .006$) and 4.60 (95% CI, 1.67–12.64; $p = .003$), respectively. The advantage of females was confirmed (OR, 4.11; 95% CI, 1.68–10.8; $p = .002$). On the contrary, arylesterase activity and paraoxonase mass concentration were not significantly associated with longevity.

DISCUSSION

Recently, the genotypic analysis of healthy Italian centenarians and Northern Ireland nonagenarians has provided evidence that PON1 genotypes at the 192 locus, in particular R+ carriers, were found significantly increased in the oldest old compared to young people. This evidence supports the hypothesis that the PON1 192 variant was involved in the definition of the longevity phenotype (17,18).

In the present study, the genetic analysis of the PON1 192 variant showed evidence of a trend toward a higher frequency of PON1 192 R+ carriers in elderly persons and in nonagenarians/centenarians with respect to young people. This result is in agreement with the above mentioned data, except for the lack of statistical significance, probably due to the smaller sample size in comparison to the previous study

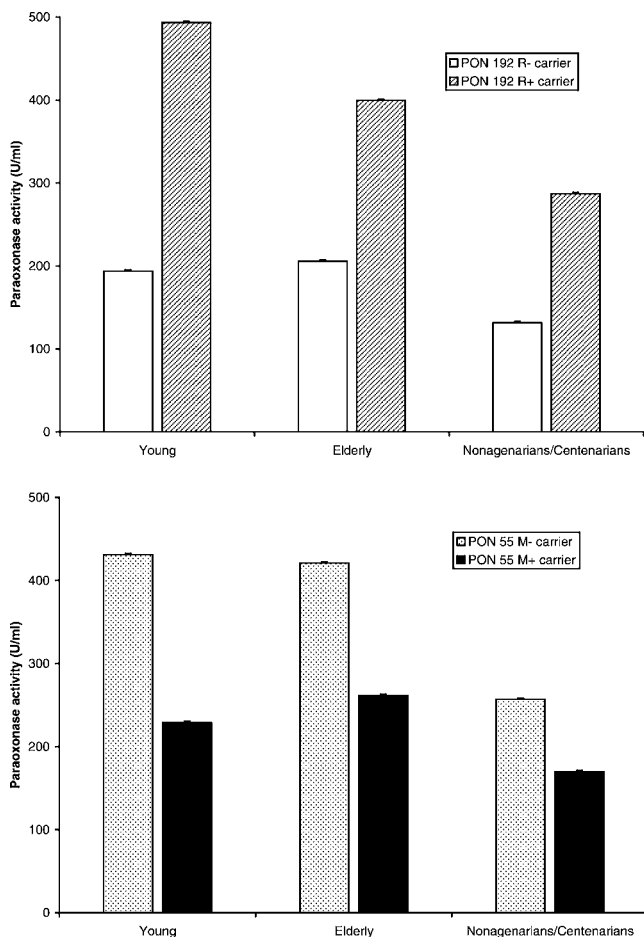


Figure 1. Mean values of paraoxonase activity among R (top) and M (bottom) carriers in the three age groups.

and for the results regarding the elderly group not evaluated in the previous work (17). The main purpose of the present study was to evaluate the relationship between genetic variants of paraoxonase and its phenotype during aging.

The first new finding of this work was the maintenance throughout life and especially in nonagenarians/centenarians of the tight correlation between the genetic variability of the PON1 gene and the enzyme activity of the gene product. In particular, R+ and M- carriers maintained significantly higher paraoxonase activity levels than did the R- and M+ carriers. This genetic control of enzymatic activity was found by several authors (5,29,30). In their studies, the correlation between phenotype and genotype was investigated in middle-aged people and did not include the oldest old, that is, the group of participants that we have taken into consideration in the present study. The maintenance of the genetic control in extreme advanced age is an interesting phenomenon that we have already described for interleukin-6 protein in centenarians (31,32).

The second interesting result of this work was the decrease during aging of paraoxonase and arylesterase activities. For the paraoxonase activity the major decay was present in the nonagenarian/centenarian group, whereas the arylesterase activity started to decrease in the elderly group.

Table 4. Multinomial Logistic Regression Model for Age Groups

Age	Covariates	OR (95% CI)	p Value
Young	Arylesterase activity	1.06 (1.03–1.09)	<.001*
	Paraoxonase mass concentration	0.99 (0.97–1.01)	.264
	Paraoxonase activity	1.01 (1.00–1.01)	.008*
	Carrier192 (R-)	1.00 (Reference)	
	Carrier192 (R+)	5.74 (1.43–23.02)	.014
	Carrier55 (M+)	1.00 (Reference)	
	Carrier55 (M-)	3.08 (1.13–8.34)	.027
	Sex (M)	1.00 (Reference)	
	Sex (F)	5.93 (2.33–15.12)	<.001*
	Elderly	Arylesterase activity	1.01 (0.98–1.04)
Paraoxonase mass concentration		0.98 (0.97–1.00)	.030
Paraoxonase activity		1.01 (1.01–1.02)	<.001*
Carrier192 (R-)		1.00 (Reference)	
Carrier192 (R+)		6.25 (1.71–22.91)	.006*
Carrier55 (M+)		1.00 (Reference)	
Carrier55 (M-)		4.60 (1.67–12.64)	.003*
Sex (M)		1.00 (Reference)	
Sex (F)		4.11 (1.68–10.8)	.002*

Note: Nonagenarians/centenarians were the comparison group.

*Level of significance $\alpha < .008$ is considered significant (see Multinomial logistic regression model).

OR = odds ratio; CI = confidence interval.

Recently, Seres and colleagues (33) found a negative and significant correlation between PON1 activity and age. We confirmed their results for paraoxonase activity extending our study also to nonagenarians/centenarians. The loss of the enzymatic activity during aging is not an uncommon phenomenon that involves several biological enzymes. In particular, the inactivation of paraoxonase enzyme activity has been attributed to the presence of lipid peroxides (34,35) and to the increase of oxidative stress with age. The data presented here suggest that the decrease of PON1 enzymatic activity is correlated to an alteration of the PON1 protein rather than to a reduction in its production.

The results of the multinomial logistic regression analysis suggest that the enzymatic activity toward paraoxon is the major variable that describes the model and has a central role throughout life. In fact, the analysis indicates that paraoxonase activity contributes substantially to determining longevity when we compared the young group versus the nonagenarians/centenarians as well as the elderly group versus the nonagenarians/centenarians. Accordingly, we can hypothesize that a high paraoxonase activity, genetically determined by PON1 192 R+ carriers and PON1 55 M- carriers, could offer a cumulative benefit starting from a young age and becoming important in reaching the extreme limits of human life span. The significant role of R+ and M- carriers in the contribution to the longevity phenotype only in the last part of the life originated from the choice of a conservative multinomial logistic regression analysis. This model suggests that genetic variability contributes mostly to determining a phenotype characterized by a high paraoxonase activity. A recent study on the three-dimensional structure of the enzyme has evidenced that the amino acid R¹⁹² was an important active-site residue. This peculiar feature might justify the alterations in the enzymatic activity; thus, it might explain the genotype-dependent variation observed in our study (36).

In previous studies, Jarvik and colleagues (14) and Mackness and colleagues (16), independently, have also

found that paraoxonase activity is a better predictor of cardiovascular disease than are PON1 192 and PON1 55 genotypes alone. In particular, they concluded that the quality of the PON1 enzyme was more important than the genetics of the PON1 gene. Here, we show that PON1 activity and genetics are deeply interconnected throughout life and that both play a role in human longevity.

ACKNOWLEDGMENTS

This work was supported in part by a grant from Italian Health Ministry fund "Progetto Finalizzato 2002 Studio Multicentrico sui Determinanti Genetici e non Genetici di Salute nell'Età Avanzata," and European Union Grants GEHA (contract LSHM-CT-2004-503270), Emilia Romagna Project "ER-GenTech," FP6 EU Project "T-CIA," and by University of Bologna "Pallotti" research funds. Richard W. James was supported by the Swiss National Research Foundation.

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Received July 27, 2005

Accepted October 31, 2005

Decision Editor: James R. Smith, PhD