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LL-Paraoxonase Genotype Is Associated with a More Severe Degree of Homeostasis Model Assessment IR in Healthy Subjects

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The LL genotype among subjects with paraoxonase (PON) polymorphism Met-Leu 54 has been shown to be associated with elevated risk of coronary heart disease. Indeed, insulin resistance (IR) is a well known cardiovascular risk factor that is likely attributable to a genetic background, lifestyle, and environmental factors such oxidative stress. Because subjects sharing the LL genotype have a more elevated degree of oxidative stress, one cannot rule out that in those subjects a more severe degree of IR can occur. Thus, the possible relationship between PON gene polymorphism and degree of IR was investigated.

In 213 healthy subjects, the degree of IR was assessed by the homeostasis model assessment, and the Met-Leu 54 PON polymorphism was detected.

The frequency was 0.366 for the LL genotype, 0.469 for the LM genotype, and 0.164 for the MM genotype. Comparing the three genotype groups, LL genotype had the more severe degree of IR, compared with LM ($P < 0.01$) and MM ($P < 0.01$) genotypes. No difference between LM and MM genotypes was found ($P = 0.49$). Subjects carrying the LL genotype were associated with the IR syndrome picture more than individuals carrying the M allele because they were more overweight and had the highest levels of triglycerides and blood pressure and the lowest values of plasma high-density lipoprotein cholesterol. In a multivariate stepwise regression analysis, LL genotype was a significant predictor of IR, independent of age, sex, body mass index, fasting plasma triglycerides, and high-density lipoprotein cholesterol ($P < 0.001$).

In conclusion, the presence of LL PON genotype is associated with a more severe degree of IR. Thus, IR might be the possible missing link between Met-Leu 54 PON polymorphism and the increased cardiovascular risk. (*J Clin Endocrinol Metab* 87: 222–225, 2002)

THE LL GENOTYPE AMONG subjects with paraoxonase (PON) polymorphism Met-Leu 54 has been shown to be associated with elevated risk of coronary heart disease (1, 2). The reason for such association lies in the fact that subjects sharing the LL genotype are less protected against oxidative stress and more exposed to the development of atherosclerosis (3–5). Interestingly, the antioxidant effect of PON enzyme activity is lower in subjects with PON LL genotype than in those carrying the M allele (1). Furthermore, LL genotype has been found associated with the carotid arteria wall thickness in subjects with familial hypercholesterolemia, resulting as an additional risk factor for carotid atherosclerosis (6).

Insulin resistance (IR) is a well known cardiovascular risk factor (6) that is able to act throughout the different ages. More specifically, IR has been shown to predict multiple atherogenic changes in lipoprotein (7) and to be causally related to coronary vascular endothelial cell dysfunction (8). Why IR develops in healthy subjects is still not fully understood. Genetic background (9), lifestyle (10), environmental factors (10), and oxidative stress may have a role (11).

Several recent studies have shown that an elevated degree of oxidative stress may impair insulin action (11), whereas IR/hyperinsulinemia *per se* can worsen the degree of oxidative stress (11). Thus, a vicious circle between oxidative stress and IR seems to be operative.

Because subjects sharing the LL genotype have a more elevated degree of oxidative stress (1), one cannot rule out that in those subjects a more severe degree of IR can occur. Thus, the degree of IR might be the missing link between the genotype (LL) and the phenotypic expression (coronary heart disease).

In light of such evidence, we aimed at investigating the degree of IR in a population with a wide age range categorized according to the PON Met-Leu 54 polymorphism.

Materials and Methods

Study protocol

Two hundred thirteen subjects (89 males and 124 females; mean age, 61.3 ± 27.5 yr) volunteered after giving informed consent. All individuals were Caucasians and were living in southern Italy. Only 67 subjects were light smokers. None was hypertensive or had clinical signs or family history of coronary heart diseases. According to American Diabetes Association criteria (12), all subjects were neither diabetics nor affected by impaired fasting glucose. All subjects were studied after overnight fast (at least 12 h). Weight and height were measured by standard techniques. Body mass index (BMI) was calculated as weight divided by height squared. All subjects had their degree of IR assessed by the homeostasis model assessment (HOMA) method (13). Baseline blood pressure was recorded by standard mercury sphygmomanometer.

The study was approved by the Ethical Committee of our institutions.

Abbreviations: BMI, Body mass index; CV, coefficient of variation; HDL, high-density lipoprotein; HOMA, homeostasis model assessment; IR, insulin resistance; LDL, low-density lipoprotein; PON, paraoxonase.

TABLE 1. Clinical characteristics of study groups (n = 213)

	LL (n = 78)	LM (n = 100)	MM (n = 35)	*P* for trend among the groups
Sex (male/female)	37/41	41/59	11/24[a]	
Age range (yr)	20–102	20–105	18–101	0.25
BMI (kg/m^2)	25.5 ± 2.3	24.9 ± 2.2	24.7 ± 1.9	<0.05
Waist/hip ratio	0.84 ± 0.04	0.83 ± 0.05	0.83 ± 0.07	0.18
Plasma glucose (mmol/liter)	5.9 ± 1.0	5.6 ± 1.1	5.4 ± 1.1	<0.05
Plasma insulin (pmol/liter)	70.29 ± 20.59	61.06 ± 27.69	55.38 ± 22.01	<0.01
Plasma triglycerides (mmol/liter)	1.2 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	<0.05
Plasma total cholesterol (mmol/liter)	4.8 ± 0.8	4.8 ± 0.8	4.8 ± 0.9	0.95
Plasma HDL cholesterol (mmol/liter)	1.2 ± 0.3	1.3 ± 0.3	1.4 ± 0.4	<0.05
Plasma LDL cholesterol (mmol/liter)	3.0 ± 0.9	3.0 ± 0.8	3.0 ± 1.0	0.93
DBP (mm Hg)	72.7 ± 4.4	66.9 ± 4.5	65.5 ± 5.1	<0.01
SBP (mm Hg)	118 ± 9	114 ± 13	111 ± 13	<0.01

Values are mean ± SD.
DBP, Diastolic blood pressure; SBP, systolic blood pressure.
[a] χ^2 test, $P < 0.05$ male *vs.* female.

PON polymorphism screening

DNA was extracted from white cells according to the procedure of Sambrook *et al.* (14) from cells obtained from a fasting blood sample. The gene polymorphism (M, methionine allele; L, leucine allele) corresponding to position 54 was analyzed by restriction isotyping using the procedure of Humbert *et al.* (15).

Analytical technique

Plasma glucose was determined by glucose oxidase method (Glucose Autoanalyzer, Beckman Coulter, Inc., Fullerton, CA), whereas plasma insulin was determined by a commercial double-antibody solid phase RIA (Linco Research, Inc., St. Charles, MO) [coefficient of variation (CV), 4.8 + 0.2%; cross-reactivity with proinsulin, 0.2%]. Plasma high-density lipoprotein (HDL) cholesterol was determined according to Penttila *et al.* (16). Commercial enzymatic methods were used in the determination of serum total cholesterol (Monotest, Boerhinger Mannheim, Milan, Italy; CV, 3.6 + 0.7%) (17) and triglyceride concentrations (Peridecrome, Boerhinger Mannheim; CV, 4.3 + 0.5%) (18).

Statistical analysis

All data were presented as means ± SD. To approximate normal distributions, plasma triglycerides, insulin, and IR (HOMA) were logarithmically transformed and used in all calculations. Allelic and genotype frequencies were compared for investigating the Hardy-Weinberg equilibrium model by the χ^2 test. One-way ANOVA with Scheffè test was used to analyze differences in clinical and laboratory findings among LL and the other genotype groups. Stepwise multivariate analysis allowed us to investigate the independent contribution of age, gender (male = 0, female = 1), BMI, fasting plasma glucose, triglycerides, HDL cholesterol, diastolic blood pressure, and PON gene polymorphism (LL = 1, LM/MM = 0) on degree of IR. A cluster analysis allowed us to evaluate whether an overall variable obtained by clustering variables of the IR syndrome was associated with PON polymorphism. For this purpose, we created a compound score referred to as a clustering score, as the sum of z-scores of the main variable of IR, BMI, triglycerides, HDL cholesterol, and diastolic blood pressure. A z-score indicates the position of an individual value of a variable in the total distribution of the variable in the population and is calculated as follows: (individual value − mean value)/SD. The association between this variable and PON polymorphism was studied using linear regression. ANOVA was used to calculate differences in the clustering analysis score of IR variable among the different genotype groups. A $P < 0.05$ was chosen for levels of significance. Statistical analyses were performed using SPSS software package (SPSS, Inc., Chicago, IL). All values are presented as means ± SD.

Results

All subjects were adult, not obese, and normotensive (Table 1). Analysis of the distribution of the genotypes revealed that the frequency was 0.366 for the LL genotype, 0.469 for the LM genotype, and 0.164 for the MM genotype. When tested, such distribution was compatible with the Hardy-Weinberg equilibrium. Comparing anthropometric and clinical characteristics among the three genotype groups (Table 1), there was a trend toward the occurrence of a more elevated BMI, plasma triglycerides, and arterial blood pressure and lower plasma HDL concentration in LL genotype than in LM and MM genotype groups. Furthermore, a more severe degree of IR was also observed in the LL genotype group compared to either LM or MM genotype groups (Fig. 1). Such difference persisted after adjustment for BMI and plasma triglycerides ($P < 0.03$). Because the subjects carrying the M allele had similar phenotypic pattern, the subjects were grouped into M non-carriers (LL) and M carriers (MM and LM). Comparing these two groups (Table 2), we found that LL genotype was associated with the IR syndrome picture. In particular, this latter group of subjects were overweight and had the more elevated levels of triglycerides and blood pressure, the more severe degree of IR, and the lowest values of plasma HDL-cholesterol levels. Furthermore, the clustering analysis score of IR variables was significantly associated with PON polymorphism (r = 0.31; $P < 0.01$). Indeed, a significant difference in the clustering analysis score of IR variable ($P < 0.001$) was found only

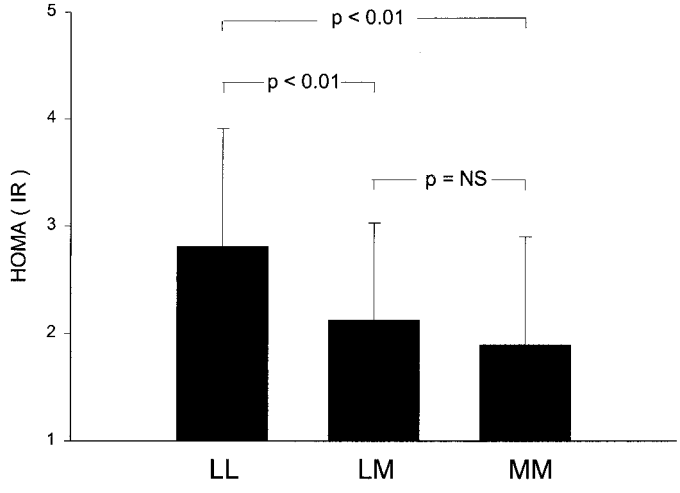

FIG. 1. Degree of IR among the three different genotype groups.

TABLE 2. Clinical characteristics of M and no-M (LL) carriers

	LL (n = 78)	M carriers (LM + MM) (n = 135)	*P*
Sex (male/female)	37/41	52/83[a]	–
Age range (yr)	20–102	18–105	NS
BMI (kg/m^2)	25.5 ± 2.3	24.8 ± 2.1	0.03
Waist/hip ratio	0.84 ± 0.04	0.83 ± 0.06	0.08
Plasma glucose (mmol/liter)	5.9 ± 1.0	5.6 ± 1.1	<0.05
Plasma insulin (pmol/liter)	70.29 ± 20.59	59.64 ± 26.27	<0.01
Plasma triglycerides (mmol/liter)	1.2 ± 0.4	1.0 ± 0.3	<0.05
Plasma total cholesterol (mmol/liter)	4.8 ± 0.8	4.8 ± 0.8	NS
Plasma HDL cholesterol (mmol/liter)	1.2 ± 0.3	1.4 ± 0.4	<0.03
Plasma LDL cholesterol (mmol/liter)	3.0 ± 0.9	3.0 ± 0.8	NS
IR (HOMA)	2.8 ± 1.1	2.0 ± 0.9	<0.03
DBP (mm Hg)	72.7 ± 4.4	66.6 ± 4.7	<0.001
SBP (mm Hg)	118 ± 9	113 ± 13	<0.01

Values are mean ± SD.
DBP, Diastolic blood pressure; SBP, systolic blood pressure.
[a] χ^2 test, $P < 0.01$ male *vs.* female.

between LL and the other genotypes, whereas no difference comparing LM and MM genotypes ($P = 0.16$) was found. Multivariate linear regression analysis with degree of IR as the dependent variable allowed us to investigate the independent contribution of LL genotype on degree of IR. In the whole population (n = 213), a model made by age, sex, BMI, fasting plasma triglycerides, HDL cholesterol, and PON genotype (LL *vs.* LM/MM) explained 53% of IR variability. In such a model, LL genotype ($P < 0.03$) was independently and significantly associated with the degree of IR (Table 3).

TABLE 3. Linear multiple regression analysis with insulin resistance as dependent variable

Variables	t	*P*
Age	1.94	0.05
BMI	2.98	0.003
Sex	−1.38	0.16
Plasma triglycerides[a]	8.10	<0.001
HDL cholesterol	−2.82	0.05
LL *vs.* LM + MM genotypes	2.98	0.003

[a] Back log transformed.

Discussion

Our study demonstrates the occurrence of a more severe degree of IR and of the IR syndrome in subjects carrying the LL PON genotype compared with those carrying LM or MM genotypes.

Several (1–5, 19–21) but not all (22, 23) studies have demonstrated that PON BB and LL genotypes are risk factors for coronary heart disease. The reason for such association may lie in the antioxidant power of PON, the product of the PON genes. In fact, antioxidant effect of PON enzyme activity is lower in subjects with PON LL genotype than in those carrying the M allele. The possible pathophysiological influence of PON Leu-Met 54 gene polymorphism has been extensively investigated. Garin *et al.* (1) demonstrated in non-insulin-dependent diabetes mellitus patients that homozygosity for the L allele was an independent risk factor for coronary heart disease (1). Furthermore, this L allele was in linkage disequilibrium with the B allele (coding for Arg at position 192), and thus its occurrence favored the simultaneous presence of the two variants, thereby leading to higher activities against their two respective substrates, paraoxon and phenyl acetate. Leviev *et al.* (24) observed that variations in the circulating concentrations of the enzyme could be related to modulation of the expression of the alleles. Indeed, these authors demonstrated that the mean concentration of the mRNA coding for the L and M variants, respectively, was significantly different.

IR is a strong cardiovascular risk factor (6) that is able to act throughout the different ages (6). Why IR develops in healthy subjects is still not fully understood. Genetic background (9), lifestyle (10), and environmental factors (10) may have a role. More recently, it has been demonstrated that elevated degree of oxidative stress may also contribute to impair insulin action (11). In particular, it has been demonstrated that oxidative stress may be associated with a reduced GLUT4 exposition (25) and/or with an impairment of insulin signaling (25). Conversely, lipoic acid, an essential cofactor of the α-oxoacid dehydrogenase complexes such as pyruvate- and α-ketoglutarate dehydrogenase with antioxidant properties, has been found to increase glucose transport in muscle cells in culture by stimulating translocation of glucose transporter GLUT4 from internal pools to the plasma membrane (26, 27).

In light of such experimental evidence, we hypothesize that a more severe degree of IR might be the result of a more elevated degree of oxidative stress and the common pathophysiological link between the genetic background and the phenotypic occurrence of coronary heart disease in subjects homozygotic for the L allele. Such hypothesis, although not demonstrated by our no-replication, cross-sectional study, is strengthened by the evidence that in subjects with LL genotype, a more severe degree of IR was also associated with several other cardiovascular risk factors [elevated BMI, plasma low-density lipoprotein (LDL) cholesterol, and blood pressure, and low plasma HDL cholesterol].

In conclusion, our study demonstrated that PON LL genotype is associated with a more severe degree of IR. Such data seem particularly important in the light of the fact that, due to the well known association with cardiovascular diseases,

IR might be the missing link between Met-Leu 54 PON polymorphism and the increased risk of cardiovascular diseases.

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