Interrelationship of serum paraoxonase activity and paraoxonase genetic variants on atherosclerosis risk

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Abstract

Paraoxonase (PON1) is a calcium-dependent esterase, closely associated with high-density lipoprotein (HDL)-containing apolipoprotein AI, that has been reported to confer antioxidant properties on HDL by decreasing the accumulation of lipid peroxidation products. PON1 activity is under genetic and environmental regulation and appears to vary widely among individuals and populations. PON1 enzyme activity for paraoxon as a substrate is modulated by a number of polymorphisms at the PON1 locus in humans. One of them is the PON1-192 genetic polymorphism that comprises PON1 Q, an isoform which has a glutamine at position 192, and shows a low activity towards paraoxon hydrolysis, while the high paraoxon-activity PON1 R isoform contains an arginine at position 192. The association of PON1 activity levels with atherosclerosis in human, animal and *in vitro* studies is consistent and exciting. Therefore, there is an obvious need to know whether environmental factors can influence serum PON1. The genetic association studies for PON1 and atherosclerosis are less consistent, since the PON1 polymorphisms probably only produce an effect on coronary heart disease risk in particular subgroups of subjects in the presence of additional factors. It is particularly important to study gene-environmental interactions that may modulate phenotypic expression of PON1 in different populations.

Keywords: Atherosclerosis, myocardial infarction, paraoxonase, paraoxonase genotypes

Resum

La paraoxonasa (PON1) és una esterasa calciodepenent estretament associada a les lipoproteïnes d'alta densitat (HDL) que contenen apolipoproteïna AI, i s'ha descrit que dóna capacitat antioxidant a les HDL, amb la qual cosa disminueix l'acumulació dels lipoperòxids. L'activitat PON1 està sota control genètic i ambiental, i varia àmpliament entre individus i poblacions. L'activitat PON1 del paraoxon com a substrat és modulada per diversos polimorfismes en el locus PON1 dels humans. Un d'aquests polimorfismes és el PON1-192, que conté PON1 Q, una isoforma amb baixa activitat per la hidròlisi de paraoxon que té una glutamina a la posició 192, mentre que la isoforma R d'alta activitat conté una arginina a la posició 192. L'associació de l'activitat PON1 amb l'arteriosclerosi en estudis humans, animals i *in vitro* és prou consistent. Per tant, cal conèixer els factors que poden influenciar la PON1 sèrica. Els estudis genètics d'associació són menys consistents, probablement perquè els polimorfismes de la PON1 tenen només efecte en el risc de malaltia cardíaca coronària en grups particulars d'individus amb la presència de factors addicionals. És particularment important estudiar les interaccions gen-ambient que poden modular l'expressió fenotípica de la PON1 en diferents poblacions.

Introduction: on the physiological function of paraoxonase

Paraoxonase (PON1) is a serum enzyme synthesized by the liver [1]. PON1 has been extensively studied in the sphere of toxicology because of its capacity to hydrolyze synthetic organophosphate compounds. These include metabolic products widely used as pesticides and nerve gases such as sarin [1]. Besides the well-known protective function of the ability of PON1 to hydrolyze organophosphate nerve agents and insecticides, some years ago it was proposed that PON1 may be involved in lipid metabolism [1].We now have several pieces of evidence that support this hypothesis. PON1 is a calcium-dependent esterase closely associated with high-density lipoprotein (HDL)-containing apolipoprotein AI [1]. Several lines of evidence are emerging which demonstrate that HDL can prevent oxidation of

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low-density lipoprotein (LDL), and that some oxidized LDL phospholipids are physiological substrates for serum PON1 [2,3]. Therefore, serum PON1 may be able to offer protection against the oxidation of the LDL phospholipids and several laboratories have undertaken basic research in this important direction.

Serum paraoxonase and lipoprotein oxidation

Human and animal studies strongly support the hypothesis that oxidative modification of LDLs performs a crucial function in the pathogenesis of atherosclerosis [4]. Therefore, mechanisms preventing LDL oxidation appear to be antiatherogenic (Figure 1). In this respect, HDL-associated PON1 may be a major defence barrier against lipid peroxides from oxidized LDLs [5]. The ability of HDL to attenuate the oxidation of LDL is largely attributable to PON1 [5]. *In vivo*, PON1 may directly act on lipid peroxides or, more likely, lipid peroxides are first transferred to HDL and then destroyed by PON1 [6]. It has been shown that purified PON1 can prevent pro-atherosclerotic effects of oxidized LDL when incubated in a vascular cell culture system [7]. The most convincing evidence produced for an anti-oxidative function of PON1 comes from the lack of protection of the HDL fraction obtained from PON1 knock-out mice, compared with HDL from the wild-type mice [8]. Clearly then,

there is an obvious need to know whether environmental factors, such as diet, physical activity or therapeutic factors, can influence serum PON1 activity or protein concentrations.

Paraoxonase activity and atherosclerosis

There are some observations [9,10] relating lower PON1 activity levels in myocardial infarction (MI) patients compared to controls. These findings have been confirmed by our laboratory in one study using 280 consecutive myocardial infarction patients and 396 control subjects from the REGICOR study (Sentí M et al, unpublished work). PON1 is able to hydrolyze a number of substrates such as paraoxon and phenyl acetate; however, the physiological substrate of PON1 remains to be discovered. Uncertainties relating to whether PON1 activity, as measured by paraoxon hydrolysis, reflects the antioxidant capacity of the enzyme have recently been reported [11]. However, in addition to low PON1 activity in patients who had suffered from MI, a significant decrease in PON1 activity toward paraoxon hydrolysis has been shown in diseases with accelerated atherogenesis such as familial hypercholesterolemia [12,13] and diabetes mellitus [12,14]. Therefore, with a physiological substrate to be defined, the function of PON1 activity measured by paraoxon hydrolysis remains to be clearly understood.
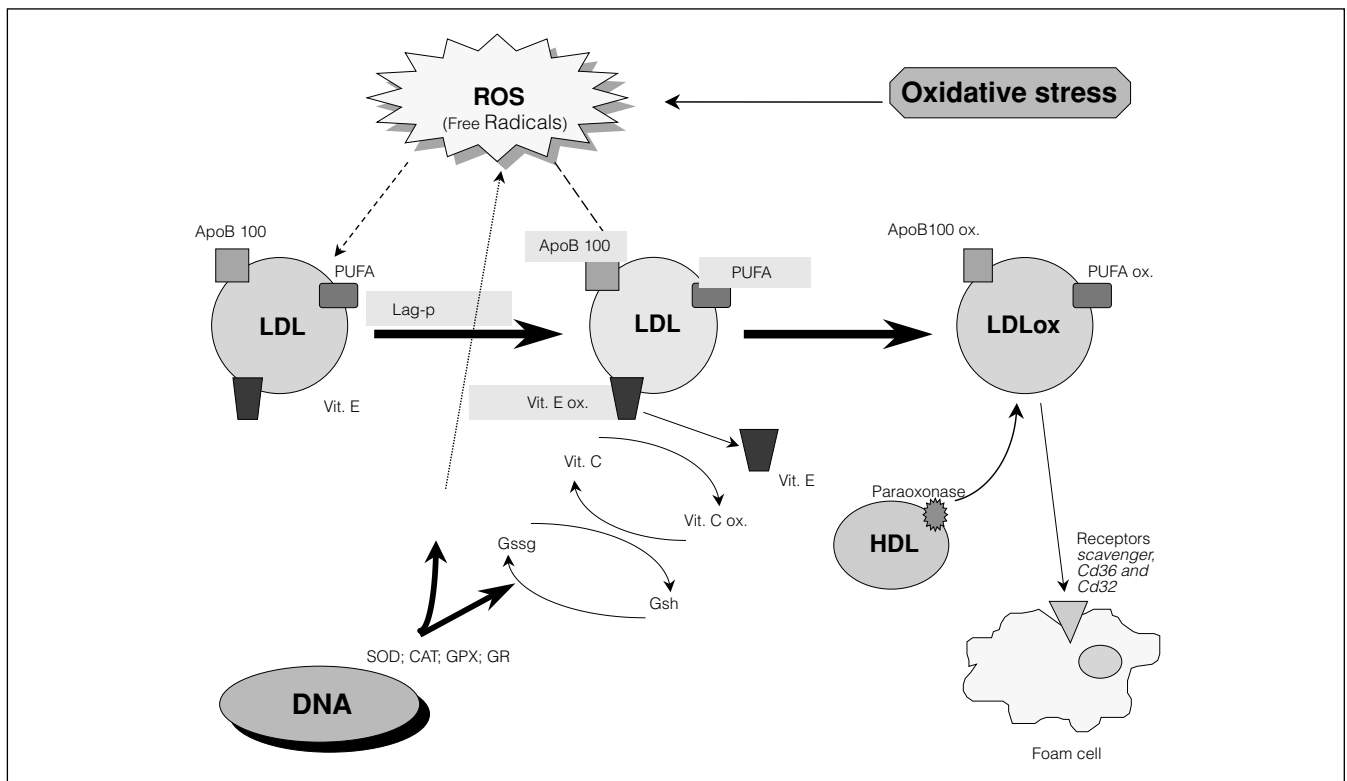

Figure 1. Relationship of the production of free radicals, lipidic peroxidation and the antioxidant systems. Free radicals generated from oxidative stress react with LDL particles, thus oxidizing vitamin E and the lipidic component of LDL. The antioxidant systems prevent LDL oxidation by reducing oxidized compounds. Apo B 100: apolipoprotein B 100; PUFA: polyunsaturated fatty acids; LDL: low density lipoproteins; E_{ox}: oxidized vitamin E; LDL_{ox}: oxidized LDL; apoB 100-100$_{ox}$: apolipoprotein B 100 oxidized; PUFA$_{ox}$: polyunsaturated fatty acids oxidized; Vit C$_{ox}$: vitamin C oxidized; Gssg: oxidized glutathion; GsH: reduced glutathion; HDL: high-density lipoprotein; SOD: superoxide dismutase; GPX: glutathion reductase; CAT: catalase.

In this respect, one point of interest concerns the inactivation of PON1 in the presence of oxidative stress. PON1 activity has been shown to be reduced in the course of oxidative incubation with Cu^{++} induced peroxidation of LDL [11]. Oxidized LDL appears to inactivate PON1 through interactions between the enzyme-free sulfhydril group and oxidized lipids which are formed during LDL oxidation [15]. There is evidence of an increase in lipid peroxidation products in patients with coronary heart disease [16]. There are also some observations suggesting that a low PON1 activity is likely to be present at the time of acute MI [10]. In this respect, PON1 activity may be partially inactivated in the presence of oxidative stress, as probably occurs in patients with coronary heart disease (CHD) or atherosclerosis. Therefore, although the possibility that PON1 decreases as a consequence of an acute phase cannot be excluded, neither can the possibility be ruled out that low PON1 activity in myocardial infarction is reflecting an increased oxidative stress.

The paraoxonase gene

PON1 activity is under genetic and environmental regulation and appears to vary widely among individuals and populations. One molecular basis of the variations in PON1 activity is a polymorphism in the PON1 gene located at position 192 of chromosome 7 which is clustered with at least two other related genes, PON2 and PON3 [17]. PON1-192 genetic polymorphism comprises PON1 Q, an isoform with low activity towards paraoxon hydrolysis, which has a glutamine at position 192, while the high-activity PON1 R isoform contains an arginine at position 192 [3]. Another polymorphism in human PON1 gene at amino acid 55, the PON1-55 polymorphism, which comprises a leucine (L) to methionine (M) substitution, seems to be more representative of PON1 protein concentration [18]. However, it has also been shown that modulates PON1 activity independently of the PON1-192 polymorphism in healthy people [18]. Individuals homozygous for the MM allele appear to have lower PON1 activity toward paraoxon compared with LL homozygotes. PON2 gene has two common polymorphisms designated G/A 148 and C/S 311 [19,20]. The genetic variant of PON2 gene at codon 148 has been found to be associated with elevated fasting plasma glucose in subjects with non-insulin-dependent diabetes mellitus [21]. Remarkably, Leviev and James [22] have recently identified three polymorphisms in the promoter region of the human PON1 gene which appear to have a great impact on PON1 activity levels and PON1 concentrations. This finding contributes to establishing a genetic basis for variations in PON1 levels, with physiological importance for the genetic nature of the antioxidant properties of HDL.

Paraoxonase1 polymorphisms and atherosclerosis

The report by Ruiz *et al* [23] was the first in a series of studies on PON1-192 gene polymorphism and CHD. Surprisingly, it was the R allele (high activity towards paraoxon) that was positively associated with the presence of CHD. Reviewing these studies reveals discrepancies even in those conducted in the same ethnic population [24,25]. In one study carried out in the USA [26], the R allele was associated with CHD, but in Europe four studies failed to show such an association [27,30]. This variability in results suggests that gene-environment and/or gene-gene interactions might modulate the relationship between PON1-192 polymorphism and CHD.

In genetic associations several geographic differences may exist and the most convincing evidence is reproducibility in different populations. We therefore conducted a case-control study in Girona, Spain, to answer the question of whether the Gln/Arg 192 PON1 polymorphism is associated with increased risk of CHD in our population and how diabetes mellitus, associated with high oxidative risk, influences such an association [31]. One hundred and fifty-six consecutive MI patients and 310 age- and sex-matched control subjects were studied. There were no differences in the distribution of genotype and allele frequencies between patients and controls. The odds ratios for diabetes and dyslipemia in control and patients stratified by genotype groups were compared. Whereas dyslipemic status was significantly related to myocardial infarction in QQ homozygotes and R carriers, diabetes mellitus was significantly associated with MI only in R-carrier subjects. In logistic regression analysis, diabetic R carriers showed more than a two and a half-fold increase in MI risk compared with nondiabetic R carriers (OR: 2.65, P<0.05). These data indicate that the R allele of the PON1-192 polymorphism is not an independent risk factor for MI in our population. However, there is an interaction between this polymorphism and diabetes mellitus consisting of increased MI risk in diabetic patients with the R allele.

There are several possible explanations for disparities among studies. First, admixture of genetically heterogeneous populations could explain some contradictory results, but it cannot explain the differences found in studies conducted in the same ethnic population. Second, discrepancies among studies may be due to a bias in patient selection. Third, PON1-192 polymorphism may reflect an underlying unknown mutation responsible for cardiovascular risk located in the same gene or in others. It is also conceivable that PON1-192 polymorphism only produces an effect on coronary heart disease risk among particular subgroups of subjects in the presence or absence of additional factors. In this respect, the possible deleterious effect of PON1-192 polymorphism may be overexpressed when a particular genetic variant and a particular oxidative condition coexist. If this is true, it would be necessary to analyze the effect of PON1-192 polymorphism on different strata of anthropometric, life style and other environmental or metabolic factors. We have conducted some studies on the relationship of increased oxidative conditions, such as smoking and aging, and altered metabolic conditions, such as HDL-deficiency status and PON1-192 polymorphism on MI risk.

Low HDL concentration is firmly established as one of the principal cardiovascular risk factors. PON1 may be responsible for part of the anti-oxidant properties of this lipoprotein. Since HDL particle is strongly associated with PON1, this lipoprotein emerges as a firm candidate to be analyzed in relation to PON1 activity and PON1-192 genotypes. The association of PON1 with HDL suggests that impaired serum concentrations of the lipoprotein could have consequences for susceptibility to oxidative stress. Since PON1-192 genetic polymorphism strongly influences PON1 activity towards paraoxon, we tested the hypothesis that this polymorphism may modulate the MI risk associated with low HDL-cholesterol concentrations (Sentí M et al, unpublished work). Two hundred and eighty consecutive MI patients and 396 control subjects were studied. To determine whether the HDL-deficiency status was differentially associated with MI risk among PON1-192 genotypes, logistic regression analyses were performed in the overall group of the study subjects and in each genotype (Figure 2). Models were adjusted for age, sex, diabetes mellitus, hypertension and smoking. In the overall population, decreased HDL cholesterol concentration conferred a MI risk of 2.56 (P=0.0001) compared to normal HDL levels. The risk increased to 4.51 (P<0.0001) in QQ homozygous HDL-deficient subjects relative to QQ homozygotes with normal HDL levels, decreased to 1.83 (P=0.1046) in QR heterozygote HDL-deficient subjects and also lowered to 1.41 (P=0.6243) in RR homozygote HDL-deficient individuals compared to RR carriers with normal HDL-cholesterol concentration. Therefore, the effect of PON1-192 genotypes on the association of low HDL-cholesterol levels with MI was gene-dosage related. These observations lead to an interesting hypothesis: PON1-192 genetic polymorphism modulates the non-fatal MI risk associated with decreased HDL cholesterol levels. The risk of non-fatal MI is increased in HDL-deficiency states principally among subjects carrying the low paraozon-activity QQ PON1-192 genotype.

Some lines of evidence indicate that with advancing age there are inflammatory modifications of the arterial wall and increased susceptibility to oxidative stress in the suben-

dothelial space which are likely to accelerate and increase severity of atherosclerosis in elderly subjects [32]. Results are controversial, but if protection by PON1 against LDL oxidation is affected by the PON1-192 polymorphism, it is conceivable that age-related myocardial infarction (MI) risk would be of different magnitude in subjects carrying the Q allele compared to those with the R allele. We conducted a study to determine whether PON1 activity and PON1-192 genetic variants have a different impact on MI risk among MI patients and controls stratified by tertiles of age distribution: young subjects ″50 years), middle-aged individuals (51 to 62 years) and subjects aged 63 to 74 (Sentí M et al, unpublished work). It is noteworthy that a decline in PON1 activity levels with advancing age was observed in subjects of both study groups carrying the low-activity QQ genotype, particularly in MI patients. We found that PON1 activity levels and age negatively correlated only in patients, and that the magnitude of this negative association was higher in MI patients carrying the more prevalent QQ and QR genotypes than in controls carrying the same genotypes. We also found that homozygote subjects for the Q allele aged over 62 showed a more than four-fold increase in MI risk compared to younger QQ homozygotes, and also with respect to older RR homozygotes. The effect of genotypes on the association of the oldest age-category with MI risk was gene-dosage related. The effect was highest in QQ genotype, intermediate in the QR genotype, and lowest in the RR genotype. Our observations lead to the following reasoning: the QQ genotype, which is associated with low PON1 activity, may be adequate to prevent lipid peroxidation in young individuals or in older subjects without atherosclerosis. However, the low-activity QQ genotype may have its antioxidant capacity exceeded when there is a coexistence of advanced age, which probably compromises PON1 function, and coronary heart disease, which still inactivates more PON1 activity.

Smokers seem to have great susceptibility to low-density lipoprotein oxidation, which probably contributes to their increased risk of atherosclerosis [33]. We investigated whether smoking, assessed by the cigarette packs smoked per year, has a differential effect related to PON1-192 genotypes on myocardial infarction (MI) risk in a case-control study (34). As expected, smokers or ex-smokers of the higher tertiles of cigarette packs smoked per year showed a significantly increased MI risk. The odds ratios for the effect of smoking on MI risk were then calculated in subjects stratified by the PON1-192 genotype groups. Whereas categorized cigarette packs smoked per year were not related to increased MI risk in R carriers, in QQ homozygotes they were significantly associated with an increased MI risk (Figure 3). This was higher among those in the higher tertile of cigarette packs smoked per year compared to those of the lower tertile (P for trend <0.001). The effects observed appear to show links between smoking, the genotype QQ of the PON1-192 polymorphism and increased risk of MI. Since PON1 is thought to exert an antioxidant effect and smoking is prone to oxidative stress, the risk of MI associated with smoking may be increased in subjects homozygous for the
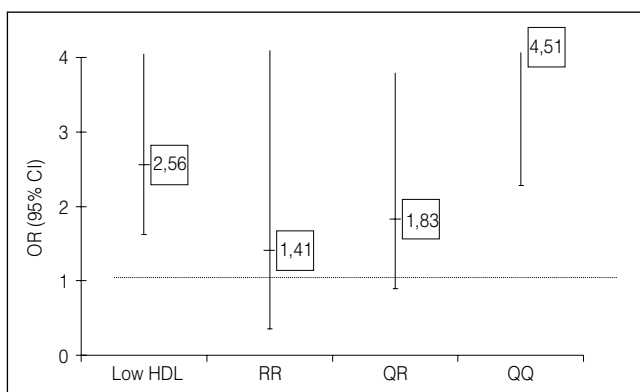

Figure 2. Adjusted odds ratios for the effect of low HDL cholesterol concentrations on myocardial infarction risk in the overall group of the study subjects and in subjects stratified by PON1-192 genotypes. Models adjusted for diabetes, hypertension, age, sex and smoking.
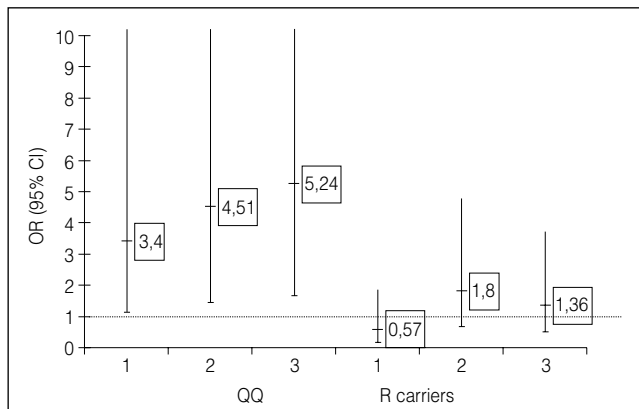
Figure 3. Odds ratios of myocardial infarction for tertiles of cigarette packs smoked per year in subjects stratified by PON1-192 genotypes.

low-activity PON1 QQ genotype. This increased MI risk appears to be time- and dose-dependent.

Some authors have failed to find associations between the variation in PON1 gene and changes in lipoprotein concentrations [27]. However, others have found significant associations of PON1-192 genetic variants with changes in HDL-cholesterol levels and in triglyceride concentrations in a relatively genetically-isolated population [35,36]. Since these changes are also characteristic of physical activity, and because it is particularly important to study gene-environmental interactions that may modulate phenotypic expression of PON1 in different populations, we attempted to ascertain whether an interaction which modifies serum lipoproteins exists between PON1-192 genetic polymorphism and physical activity [37]. Overall, our results suggested no influence of PON1-192 genetic variation on lipids and lipoproteins. However, physical activity was associated with increased HDL-cholesterol levels and decreased triglyceride levels only in men carrying the R allele (Figure 4). R carriers included in the lower physical activity tertile had an HDL-cholesterol mean concentration significantly
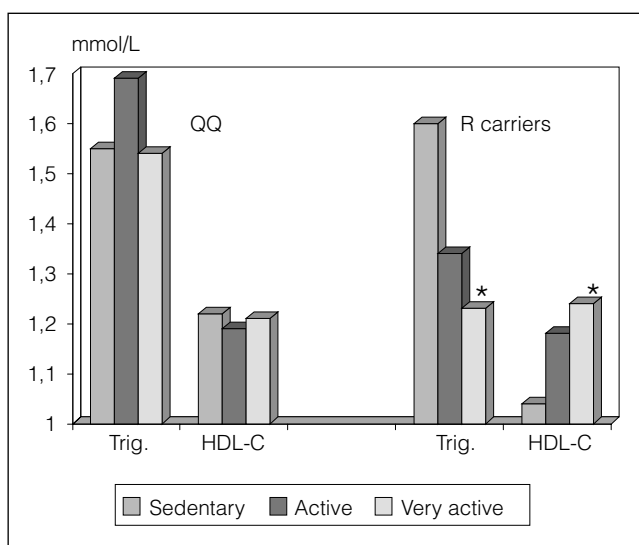

Figure 4. Serum triglycerides and HDL-cholesterol in men stratified by the amount of physical activity and PON1-192 genotypes. *Significantly different from sedentary subjects, $P < 0.001$.

lower than QQ homozygous men in the same physical activity category. In contrast, R carriers of the second tertile achieved HDL-cholesterol levels similar to those of QQ subjects and, interestingly, better serum triglycerides. Very active R carriers showed the most favorable lipid profile in this genotype group. Therefore, the existence of a single R allele seems to strongly impair the lipid profile, particularly HDL-cholesterol concentrations, in men if physical activity is not regularly undertaken, but our results also suggest that with moderate increases in physical activity the beneficial effect on lipids is already obtained in subjects with the R allele.

Conclusions

PON1 is an HDL-associated enzyme that protects lipoproteins from oxidative modifications. PON1, which hydrolyzes organophosphates such as paraoxon, appears to use phospholipids on LDL as a physiological substrate. The association of PON1 activity levels with atherosclerosis in human, animal and *in vitro* studies is consistent and exciting. Therefore, there is an obvious need to know whether environmental factors can influence serum PON1. The genetic association studies for PON1 are less consistent, since probably the PON1 polymorphisms only produce an effect on coronary heart disease risk among particular subgroups of subjects in the presence of additional factors. Until now, in those studies in which an association of the PON1-192 polymorphism with coronary heart disease was observed, it was paradoxically the high paraoxon-activity R allele that was associated. However, we recently reported that the risk of MI associated with a classical risk factor such as smoking may be increased in subjects homozygous for the low paraoxon-activity PON1 QQ genotype. Furthermore, we recently found that the QQ genotype may represent an additional risk factor for MI in subjects with low HDL cholesterol levels.

Much more remains to be learnt about the possible relevance of the PON1 gene and its product. It is conceivable that an antioxidant diet, which seems to perform a crucial function in the lower prevalence of coronary heart disease in the Mediterranean area, may influence PON1 activity. It is also particularly important to study gene-environmental interactions that may modulate phenotypic expression of PON1 in different populations.

Acknowledgements

References

[1]   Mackness MI. Possible medical significance of human serum paraoxonase. 1989. In: Reiner E, Aldridge WN, Hoskin FCG, eds. Enzymes Hydrolysing Organopho-

sporus Compounds. Chichester, UK: Ellis-Horwood, pp:202-213.

[2] Mackness MI, Arrol S, Durrington PN. 1991. Paraoxonase prevents accumulation of lipoperoxides in low-density lipoprotein. FEBS Lett 286:152-154.

[3] Mackness MI, Mackness B, Durrington PN, Connelly PW, Hegele RA. 1996. Paraoxonase: biochemistry, genetics and relationship to plasma lipoproteins. Curr Opin Lipidol 7:69-76.

[4] Berliner JA, Heinecke JW. 1996. The role of oxidized lipoprotein in atherogenesis. Free Radic Biol Med 20:707-727.

[5] Mackness MI, Arrol S, Abbott CA, Durrington PN. 1993. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis 104:129-135.

[6] Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. 1998. Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. J Clin Invest 101:1581-1590.

[7] Watson AD, Berliner JA, Hama SY, La Du BN, Fault KF, Fogelman AM, Navab M. 1995. Protective effect of high density lipoprotein associated paraoxonase-inhibition of the biological activity of minimally oxidised low-density lipoprotein. J Clin Invest 96:2882-2891.

[8] Shih DM, Gu L, Xia YR, Navab M, Li WF, Hama S, Castellani LW, Furlong CE, Costa LG, Fogelman AM, Lusis AJ. 1998. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 394:284-287.

[9] Mc Elveen J, Mackness MI, Colley CM, Peard T, Warner S, Walker CH. 1986. Distribution of paraoxon hydrolytic activity in the serum of patients after myocardial infarction. Clin Chem 32:671-673.

[10] Ayub A, Mackness MI, Arrol S, Mackness M, Patel J, Durrington PN. 1999. Serum paraoxonase after myocardial infarction. Arterioscler Thromb Vasc Biol 19:330-335.

[11] Cao H, Girard-Globba A, Berthezene F, Moulin P. 1999. Paraoxonase protection of LDL against peroxidation is independent of its esterase activity towards paraoxon and is unaffected by the Q→R genetic polymorphism. J Lipid Res 40:133-139.

[12] Mackness MI, Harty D, Bhatnagar D, Winocour PH, Arrol S, Ishola M, Durrington PN. 1991. Serum paraoxonase activity in familial hypercholesterolemia and insulin-dependent diabetes mellitus. Atherosclerosis 86:193-199.

[13] Tomàs M, Sentí M, García-Faria F, Vila J, Torrents A, Covas M, Marrugat J. 2000 Effect of simvastatin therapy on paraoxonase activity and related lipoproteins in familial hypercholesterolemic patients. Arterioscler Thromb Vasc Biol, 20:2113-2119.

[14] Mackness B, Mackness MI, Arrol S, Turkie W, Julier K, Abuasha B, Miller JE, Boulton AJM, Durrington PN. 1998. Serum paraoxonase (PON1) 55 and 192 polymorphism and paraoxonase activity and concentration in non-insulin dependent diabetes mellitus. Atherosclerosis 139:341-349.

[15] Aviram M, Rosenblat M, Billecke S, Erogul J, Sorenson R, Bisgaier ChL, Newton RS, La Du BN. 1999. Human serum paraoxonase (PON1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radic Biol Med 26:892-904.

[16] Liu K, Cuddy TE, Pierce GN. 1992. Oxidative status of lipoproteins in coronary disease patients. Am Heart J 123:285-289.

[17] Primo-Parmo SL, Sorenson RC, Teiber J, La Du BN. 1996. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. Genomics 33:498-507.

[18] Garin MC, James RW, Dussoix P, Blanche H, Passa P, Froguel P, Ruiz J. 1997. Paraoxonase polymorphism Met-Leu54 is associated with modified concentrations of the enzyme. A possible link between the paraoxonase gene and increased risk of cardiovascular disease in diabetes. J Clin Invest 99:62-66.

[19] Mochizuki H, Scherer SW, Xi T, Nickle DC, Majer M, Huizenga JJ. 1998. Human PON2 gene at 7q21.3: cloning, multiple mRNA forms, and missense polymorphisms in the coding sequence. Gene 213:149-157.

[20] Sanghera DK, Aston CE, Saha N, Kamboh MI. 1998. DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. Am J Hum Genet 62:36-44.

[21] Hegele RA, Connelly PW, Scherer SW, Hanley AJG, Harris SB, tsui LC, Zinman B. 1997. Paraoxonase-2 gene (PON2) G148 variant associated with fasting plasma glucose in non-insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 82:3373-3377.

[22] Leviev I, James RW. 2000. Promoter polymorphisms of human paraoxonase PON1 gene and serum paraoxonase activities and concentrations. Arterioscler Thromb Vasc Biol 20:516-521.

[23] Ruiz J, Blanche H, James RW, Blatter Garin M.-C, Vaisse C, Charpentier G, Cohen N, Morabia A, Passa P, Froguel P. 1995. Gln-Arg 192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. Lancet 346:869-872.

[24] Suehiro T, Nakauchi Y, Yamamoto M, Arii K, Itoh H, Hamashige N, Hashimoto K. 1996. Paraoxonase gene polymorphism in Japanese subjects with coronary heart disease. Int J Cardiol 57:69-73.

[25] Zama T, Murata M, Matsubara Y, Kawano K, Aoki N, Yoshino H, Watanabe G, Ishikawa K, Ikeda Y. 1997. A [192]Arg variant of the human paraoxonase (HUMPONA) gene polymorphism is associated with an increased risk for coronary artery disease in the Japanese. Arterioscler Thromb Vasc Biol 17:3565-3569.

[26] Serrato M, Marian AJ. 1995. A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. J Clin Invest 96:3005-3008.

[27] Antikainen M, Murtomäki S, Syvänne M, Pahlman R, Tahvanainen E, Jauhiainen M, Frick MH, Ehnholm C. 1996. The Gln-Arg191 polymorphism of the human paraoxonase gene (HUMPONA) is not associated with the risk of coronary heart disease in Finns. J Clin Invest 98:883-885.

[28] Rice GI, Ossei-Gerning N, Stickland MH, Grant PJ. 1997.The paraoxonase Gln-Arg 192 polymorphism in subjects with ischaemic heart disease. Coron Artery Dis 8:677-682.

[29] Herrmann S-M, Blanc H, Poirier O, Pahlman R, Tah-vanainen E, Jauhiainen M, Frick MH, Ehnholm C. 1996. The Gln/Arg polymorphism of human paraoxonase (PON 192) is not related to myocardial infarction in the ECTIM Study. Atherosclerosis 126:299-303.

[30] Ombres D, Panniteri G, Montali A, Candeloro A, Secca-reccia F, Campagna F, Cantini R, Campa PP, Ricci G, Arca M. 1998. The Gln-Arg192 polymorphism of hu-man paraoxonase gene is not associated with coro-nary artery disease in Italian patients. Arterioscler Thromb Vasc Biol 18:1611-1616.

[31] Aubó C, Sentí M, Marrugat J, Tomàs M, Vila J, Sala J, Masiá R. 2000. Risk of myocardial infarction associated with Gln/Arg 192 polymorphism in human paraoxonase gene and diabetes mellitus. Eur Heart J 21:33-38.

[32] Bilato C, Crow MT. 1996. Atherosclerosis and the vas-cular biology of aging. Aging 8:221-34.

[33] Harats D, Ben Naim M, Dabach Y, Hollander G, Stein O, Stein Y. 1989. Cigarette smoking renders LDL sus-ceptible to peroxidative modification and enhanced metabolism by macrophages. Atherosclerosis 79:245-252.

[34] Sentí M, Aubó C, Tomàs M. 2000 Differential effects of smoking on myocardial infarction risk according the Gln/Arg variants of the human paraoxonase gene. Me-tabolism, 49:557-559.

[35] Hegele RA, Brunt JH, Connelly PW. 1995. A polymor-phism of the paraoxonase gene associated with varia-tion in plasma lipoproteins in a genetic isolate. Arte-rioscler Thromb Vasc Biol 15:89-95.

[36] Boright AP, Connelly PW, Brunt JH, Scherer SW, Tsui L-C, Hegele RA. 1998. Genetic variation in paraox-onase-1 and paraoxonase-2 is associated with varia-tion in plasma in Alberta Hutterites. Atherosclerosis 139:131-136.

[37] Sentí M, Aubó C, Elosua R, Sala J, Tomàs M, Marrugat J. 2000. Effect of physical activity on lipid levels in a population-based sample of men with and without the Arg192 variant of the human paraoxonase gene. Genet Epidemiol 18:276-286.

About the authors

The Unitat de Lipids i Epidemiologia Cardiovascular *of the IMIM was formed in 1994. In that year, coinciding with an internal reorganization of the IMIM, a group of epidemiologists, biochemists, cardiologists, intensive care specialists, internists, biosta-tisticians and vascular surgeons who were working parallel on the study of different aspects of arteriosclerosis and its bio-chemical bases, risk factors, clinical consequences and epidemiology in Spain, teamed up to approach the problem of cardio-vascular diseases collaboratively. This multidisciplinary focus provided an approach from various perspectives which enriched the study of a multifactorial process such as arteriosclerosis.*

In 1996, a new line of research in cardiovascular genetic epidemiology was started up in the Molecular Biology laboratory of the Unit. Its main objective is to study the interaction of genetic and environmental factors, with special emphasis on the search for protective factors against cardiovascular disease. In this respect, a case-control study was designed, which, in addition to one matched for age and sex, included a control group of very elderly healthy subjects. The aim of this study was to identify predisposing factors for longevity free from cardiovascular disease, without the inconvenience of studies with age-matched controls in which the subjects who are apparently healthy may develop the disease in the future. Its interest is centred on the genetic polymorphisms related to paraoxonase, an enzyme with antioxidant capacity bound to high-density lipoproteins. An-other major point of interest concerns the study of the association between certain environmental factors such as physical ac-tivity and diet and genetic determinants of serum paraoxonase.