

The Gln-Arg191 Polymorphism of the Human Paraoxonase Gene (HUMPONA) Is Not Associated with the Risk of Coronary Artery Disease in Finns

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Abstract

The human paraoxonase gene (HUMPONA) is codominantly expressed as alleles A and G. The A allele codes for glutamine (A genotype) and the G allele for arginine (B genotype) at position 191 of the paraoxonase enzyme. This genetic polymorphism has been suggested to be associated with the predisposition to coronary artery disease (CAD). We investigated the frequency of paraoxonase A and G alleles in 380 well-characterized CAD patients and in 169 controls. The most common genotype in both the patients with CAD (211/380) and in healthy Finnish individuals (87/169) was AA (Gln/Gln). The heterozygous A/B (Gln/Arg) genotype was present in 140 of the patients and in 75 controls. The frequency of the A allele was 0.74 in both patients and controls. The genotype distribution between the two groups did not differ ($P = 0.12$, χ^2 test). The genotype distributions were also similar to those reported earlier in other caucasoid populations. In conclusion, we found no association between the Gln-Arg 191 polymorphism of the human paraoxonase gene and coronary artery disease in Finns. (*J. Clin. Invest.* 1996. 98:883–885.) Key words: paraoxonase • gene, polymorphism • atherosclerosis • coronary artery disease

Introduction

There is increasing evidence that genetic factors make a prominent independent contribution to the risk of coronary artery disease (CAD).¹ One gene that has been related to the pathogenesis of CAD is the human paraoxonase gene (HUMPONA). The gene is codominantly expressed as alleles A and G. The A

allele (genotype A) codes for a paraoxonase enzyme (PONA) having glutamine at position 191, whereas the G allele (genotype B) codes for a protein with arginine at position 191 (1, 2). PONA is a Ca^{2+} -dependent high density lipoprotein (HDL)-associated ester hydrolase (EC 3.1.8.1, arylalkylphosphatase) that catalyzes the hydrolysis of toxic metabolites of organophosphates and nerve gases (3, 4). The physiological substrate is not known. Previous studies have shown that variation in PONA activity appears to be caused by polymorphism of the HUMPONA. Subjects homozygous for the A allele have lower plasma PONA activity than subjects homozygous for the G allele (5). Two recent studies in which HUMPONA allele frequencies were compared in CAD patients and in control individuals suggested that the two allelic variants of the HUMPONA are independent risk factors of coronary atherosclerosis (5, 6). It was therefore of interest to study whether this association also exists in Finnish patients with CAD.

Methods

Study population. The allele distribution of the Gln-Arg191 polymorphism was determined in 380 CAD patients and in 169 control individuals. The patients were participants of a secondary prevention trial of CAD (7). The trial enrolled Finnish men under 70 years of age who had undergone coronary bypass surgery up to 4 years before the study. Additional inclusion criteria were HDL cholesterol ≤ 1.1 mmol/liter, LDL cholesterol ≤ 4.5 mmol/liter, and serum triglyceride concentration ≤ 4.0 mmol/liter. Subjects with a history of diabetes, current regular smoking, body mass index > 30 kg/m², uncontrolled hypertension, left ventricular ejection fraction $< 35\%$, and those with significant liver, kidney, or thyroid disease were excluded.

The control individuals had no symptoms of CAD or any other disease (78 males, mean age 37 years, 91 females, mean age 36 years).

Clinical and laboratory measurements. Serum TG and cholesterol were measured by automated enzymatic methods using the Cobas Mira autoanalyzer (Hoffman-La Roche, Basel, Switzerland). HDL cholesterol was measured after precipitation of the apoB-containing lipoproteins with phosphotungstic acid and magnesium chloride. LDL cholesterol was calculated by the Friedewald formula. The data presented represent the means of three fasting samples taken during a period of 1.9 ± 1.0 (mean \pm SD) months while the subjects were advised to consume an American Heart Association Step 1 diet. 81% of the subjects were taking β -blockers, but no other drugs affecting lipoprotein metabolism were used. The study subjects in the patient group were 59.1 ± 6.8 yr old, had a body mass index of 26.4 ± 2.2 kg/m²; and serum triglyceride, serum cholesterol, HDL cholesterol, and LDL cholesterol concentrations 1.64 ± 0.64 , 5.17 ± 0.64 , 0.82 ± 0.14 , and 3.61 ± 0.53 mmol/liter, respectively.

Genotyping. The genomic DNA from white blood cells was iso-

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Received for publication 25 April 1996 and accepted in revised form 14 June 1996.

1. Abbreviations used in this paper: CAD, coronary artery disease; HUMPONA, human paraoxonase/arylesterase gene; PONA, paraoxonase/arylesterase.

J. Clin. Invest.

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0021-9738/96/08/0883/03 \$2.00

Volume 98, Number 4, August 1996, 883–885

lated according to (8). The A → G mutation at codon 191 was determined using the solid-phase minisequencing method (9). In this method variable nucleotides are identified by a single nucleotide primer extension reaction catalyzed by DNA polymerase on a solid support. The polymorphic, 99-bp DNA-fragment was amplified by PCR using the following primers: I primer 5'- (ATT GTT GCT GTG GGA CCT GAG) and the biotinylated II primer 5'- (CCA CGC TAA ACC CAA ATA CAT C). The detection primer required in minisequencing was 5'- (ATT TTC TTG ACC CCT ACT TAC).

Statistical analysis. The distribution of paraoxonase genotypes in CAD and control groups were compared by the Chi-square test of independence. The distribution of genotype frequencies was compared to the Hardy-Weinberg equilibrium model using the χ^2 test. Fisher's exact test was performed to compare allele frequencies between these groups. A published equation for sample size estimations with categorical frequency data was used to evaluate the statistical power of the study (10). One-way analysis of variance (ANOVA) was used to analyze relationship between genotypes and clinical and laboratory findings in the patient group.

Results

Genotype and allele distribution between patient and control group. The genotype and allele frequencies of the paraoxonase gene Gln-Arg191 polymorphism in patients and controls are shown in Table I. The genotype frequencies did not differ significantly between the two groups ($P = 0.12$, χ^2 test). The AA (Gln/Gln) genotype was the most common in patients with CAD (211/380) and in controls (87/169). The A/B (Gln/Arg) genotype was present in 140/380 of the patients and in 75/169 controls. The rarest genotype in both patients (29/380) and controls (7/169) was BB (Arg/Arg). The alleles G and A occurred at the same frequency of 0.26 and 0.74 in both groups ($P = 0.94$, Fisher's exact test). The noted G (Arg) allele frequency (0.26) in both groups was similar to the allele frequencies in the general US (0.31) and French (0.24) populations and similar to that previously reported for French diabetic patients without coronary heart disease (0.26) (5, 6).

The frequency distribution of genotypes was compatible with Hardy-Weinberg equilibrium when tested with the χ^2 test ($P = 0.06$, $P = 0.36$, and $P = 0.12$ in controls, CAD patients, and all study subjects, respectively). To detect a difference in allele frequencies between CAD and control groups which would be of a similar magnitude as previously reported (0.44 vs. 0.31%) (6) we estimated that counting about 289 alleles for both groups would be enough at a significance level

Table I. HUMPPONA Genotypes and the Frequency of Alleles

	Control (n = 169)	CAD (n = 380)
Genotypes*		
AA	87(52%)	211 (56%)
A/B	75 (44%)	140 (37%)
BB	7 (4%)	29 (7%)
Alleles†		
A (Gln)	0.737	0.739
G (Arg)	0.263	0.261

* $\chi^2 = 4.11$; $P = 0.128$. † $P = 0.94$.

Table II. Clinical and Laboratory Measurements in CAD Patients with Different Genotypes

	AA (n = 211)	AB (n = 140)	BB (n = 29)	ANOVA P
Age	58.5±7.0	60.4±6.2	56.7±6.4	0.006
BMI	26.6±2.3	26.1±2.1	26.4±2.3	0.114
TG	1.60±0.63	1.71±0.68	1.63±0.64	0.288
Cholesterol	5.16±0.64	5.21±0.63	5.19±0.66	0.814
HDL-C	0.82±0.14	0.81±0.14	0.83±0.16	0.832
LDL-C	3.62±0.53	3.62±0.53	3.63±0.59	0.996

$P < 0.05$ and with a power of 90% ($Z\beta = 1.28$). In this study we counted 760 alleles in the CAD group and 338 alleles in the control group.

Relations between clinical and laboratory findings and genotypes in the patient group. When clinical and laboratory values were compared among genotypes in the patient group no significant differences were noted with regard to body mass index or triglyceride, cholesterol, HDL-cholesterol or LDL-cholesterol levels (Table II). The heterozygote (A/B) group was on average slightly, but significantly, older than either homozygote group (Table II).

Discussion

Two previous studies (5, 6) concluded that the paraoxonase gene polymorphism Gln-Arg 191 is associated with a predisposition to coronary atherosclerosis. In both studies patients with coronary heart disease had an increased frequency of the G allele and the B genotype. Multiple logistic regression analysis further revealed that in both studies this association was independent of gender and conventional risk factors for CAD. The nomenclature of the polymorphism is somewhat confusing, the G allele codes for arginine at position 191 and the B genotype. In previous studies two amino acid numbering systems have been used (1, 2). In the present study we use the fixed nomenclature, in which the allele and genotype have different names, and use the numbering system in which alanine, the first amino acid of the mature protein, is number 1 and the Gln-Arg polymorphism is coded by nucleotides 574–576 in cDNA (2).

In the present study the paraoxonase allele and genotype frequencies were similar in the CAD patients and in the group of healthy Finnish subjects. Thus we found no indication of an association between the HUMPPONA polymorphism and CAD in Finns. At present we have no explanation for this observation, however, several aspects of the studies should be borne in mind when interpreting this apparent discrepancy.

One possibility is that the Finnish gene pool is such that the association is lost. The Finnish gene pool shows great genetic homogeneity at the population level (11) and the A and B allele frequencies 0.74 and 0.26 in both in patients and in the control group are similar to the published allele frequencies in the French and US populations of Caucasian origin. We therefore do not think that the explanation for the discrepancy would be at the population level or due to genetic heterogeneity within or between our study groups.

Another explanation for the discrepancy between the present study and the two previous ones could be patient selection. Serrato and Marian (6) studied patients who underwent percu-

tanous transluminal coronary angioplasty and Ruiz et al. (5) studied diabetic patients. Our CAD patients represent a selected group of survivors after coronary bypass surgery who have low HDL cholesterol levels without any other major dyslipidemia. All three studies are case-control in design and the patients represent selected groups of CAD patients. Therefore, the possibility of bias cannot be ruled out. Further prospective and family studies are needed to evaluate whether the polymorphism of the paraoxonase gene is a risk factor for CAD.

The pathophysiological mechanism underlying the putative association between the HUMPONA polymorphism and susceptibility to CAD is not known. One study has indicated a significant association between the HUMPONA polymorphism and plasma lipids, the A allele carriers having a more favorable lipid profile (12). This finding was, however, not confirmed in another study (5). In our study no indication of an effect of the HUMPONA polymorphism on plasma lipid levels could be observed. Recently it was shown that paraoxonase may protect the artery wall by destroying harmful lipid components in oxidized LDL (13). Ruiz et al. (5) observed a higher serum paraoxonase activity in G allele carriers than in A allele carriers. Thus the observation that the B genotype, which is linked to higher activity, is associated with CAD (5, 6) is surprising.

Further studies are needed to clarify whether paraoxonase is involved in atherosclerosis by preventing lipid oxidation in the circulation and whether there are other mechanisms linking paraoxonase to CAD. Further work is also warranted to study the association between paraoxonase polymorphism and CAD in different populations; we could not verify the association in a Finnish CAD patient group.

References

1. Humbert, R., D.A. Adler, C.M. Distech, C. Hassett, C.J. Omiecinski, and C.E. Furlong. 1993. The molecular basis of the human serum paraoxonase activity polymorphism. *Nature Genet.* 3:73-76.
2. Adkins, S., K.N. Gan, M. Mody, and B.N. La Du. 1993. Molecular basis for the polymorphic forms of human serum paraoxonase/arylesterase: glutamine or arginine at position 191, for the respective A or B allozymes. *Am. J. Hum. Genet.* 52:598-608.
3. La Du, B.N. 1992. Human serum paraoxonase/arylesterase. In *Pharmacogenetics of Drug Metabolism*. W. Kalow, editor. Pergamon Press, New York. 51-91.
4. Mackness, M.I. 1989. 'A'-esterases — enzymes looking for a role? *Biochem. Pharmacol.* 38:385-390.
5. Ruiz, J., H., Blanche, R.E. James, M-C.B. Garin, C. Vaisse, G. Charpentier, N. Cohen, A. Morabia, P. Passa, and P. Froguel. 1995. Gln-Arg192 polymorphism of paraoxonase and coronary heart disease in type 2 diabetes. *Lancet.* 346:869-872.
6. Serrato, M., and A.J. Marian. 1995. A variant of human paraoxonase/arylesterase (HUMPONA) gene is a risk factor for coronary artery disease. *J. Clin. Invest.* 96:3005-3008.
7. Syväne, M., M.S. Nieminen, M.-R. Taskinen, and M.H. Frick. 1994. An angiographic secondary prevention trial with gemfibrozil in post-bypass men with low plasma HDL-C concentrations: study design and baseline characteristics. *Atherosclerosis.* 100:153-154 (Abstr.)
8. Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. *Molecular cloning: a laboratory manual*. Cold Spring Laboratory Press, New York.
9. Syväne, A.C., A. Sajantila, and M. Lukka. 1993. Identification of individuals by analysis of biallelic DNA markers, using PCR and solid-phase minisequencing. *Am. J. Hum. Genet.* 52:46-59.
10. Norman, G.R., and D.L. Steiner. 1994. *Biostatistics. The bare essentials*. Mosby-Year Book, St. Louis, Missouri. 260 pp.
11. Nevanlinna, H. 1972. The Finnish population structure. A genetic and genealogical study. *Hereditas.* 71:195-236.
12. Hegele, R.A., J.H. Brunt, and P.W. Connelly. 1995. A polymorphism of the paraoxonase gene associated with variation in plasma lipoproteins in a genetic isolate. *Arterioscler. Thromb. Vasc. Biol.* 15:89-95.
13. Watson, A.D., J.A. Berliner, S.Y. Hama, B.N. La Du, K.F. Fauli, A.M. Fogelman, and M. Navab. 1995. Protective effect of high density lipoprotein associated paraoxonase. Inhibition of the biological activity of minimally oxidized low density lipoprotein. *J. Clin. Invest.* 96:2882-2891.