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## Paraoxonase 1 Polymorphisms and Risk of Myocardial Infarction in Women and Men

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### Abstract

**Background**—Previous studies of paraoxonase 1 (PON1) genetic variants and coronary heart disease (CHD) have been conflicting. However, the modifying effects of lifestyle factors that affect PON1 activity are uncertain.

**Methods and Results**—In parallel nested case-control studies, we examined the prospective associations between PON1 polymorphisms Q192R and L55M and incident CHD among participants in the Nurses' Health and Health Professionals Follow-up Studies. We followed women for 8 years and men for 6 years, and documented 249 women and 266 men with incident CHD. Neither polymorphism was associated with risk of CHD in either sex, and neither monounsaturated fat intake nor smoking interacted with genotype. Among women, there was a possible interaction of Q192R with alcohol intake (*p* interaction 0.06) and a suggestion of a similar interaction with L55M genotype (*p* interaction 0.11). In analyses of both polymorphisms, alcohol intake 2.5 gm/day was associated with lower risk among all women (odds ratio 0.45) except those with the Q192Q/L55M genotype (odds ratio 1.33; *p* 3-way interaction 0.07).

**Conclusions**—PON1 polymorphisms are not associated with risk of CHD, nor do they interact with smoking or monounsaturated fat intake. A possible gene-alcohol interaction should be considered in future studies of PON1 and CHD.

### Introduction

Paraoxonase 1 (PON1) has attracted great interest as an endogenous antiatherogenic protein. It is a hepatically-synthesized 43-kd protein tightly associated with high-density lipoprotein (HDL) particles in serum. It may play a role in cardioprotection, as it prevents low-density lipoprotein cholesterol (LDL-C) oxidation, metabolizes oxidized LDL-C, and interferes with macrophage uptake of LDL particles.(1)

Based on its diverse activities on lipids and lipoprotein particles, a number of studies have evaluated genetic variants in the PON1 gene and their association with risk of coronary heart disease (CHD) and other chronic illnesses.(2, 3) The PON1 gene is clustered in tandem with genes for PON2 and PON3 on the long arms of chromosome 7 (q21.22), and several nonsynonymous single nucleotide polymorphisms (SNPs) have been identified. Two meta-analyses have evaluated the relationship of these SNPs with CHD risk, with little evidence of a main effect for any single SNP,(3, 4) but subsequent studies continue to suggest associations with risk.(5–7)

A potential limitation of previous work on PON1 variants and CHD risk has been the absence of detailed information on diet with which to evaluate gene-environment interactions. In particular, alcohol intake is closely correlated with serum levels of HDL-C(8, 9) and increases levels of HDL-C in randomized trials.(10) In addition to its effects on HDL-C per se, alcohol intake also appears to promote PON activity directly in both human and animal experiments,(11–13) an effect that may be modified by PON1 variants in population studies.(14) Intriguingly, the Atherosclerosis Risk in Communities study(15) found that Q192R genotype significantly modified the positive association of heavy drinking with CHD, albeit only among black men and not at lower levels of intake. More limited data also suggest beneficial effects of monounsaturated fat intake(16–20) and detrimental effects of smoking(14, 21–23) on PON activity that may be modulated by PON1 variants.

To investigate the relationships of two common PON1 polymorphisms with risk of CHD, and their interactions with alcohol intake, smoking, and monounsaturated fat intake, we performed nested case-control studies of men and women enrolled in the Health Professionals Follow-up Study (HPFS) and the Nurses' Health Study (NHS), two parallel prospective cohorts of men and women in the United States.

## Methods

### Study Population

The NHS cohort was established in 1976.(24–26) The study population consists of 121,700 married female registered nurses aged 30 to 55 years residing in one of 11 larger US states. Women have received follow-up questionnaires biennially to update information on exposures and newly diagnosed illnesses. Since 1980, participants have updated information on diet, alcohol, and vitamin supplements through a food frequency questionnaire every four years.

The HPFS began in 1986, when 51,529 male health professionals 40 to 75 years of age completed the initial 6-page HPFS questionnaire.(27, 28) The population includes 29,683 dentists, 3,745 optometrists, 2,218 osteopathic physicians, 4,185 pharmacists, 1,600 podiatrists, and 10,098 veterinarians. Biennial follow-up has mirrored the NHS.

These studies have been reviewed by and received annual approval from the Harvard School of Public Health Human Subjects Committee.

### Assessment of PON1 Genotype

Blood samples were requested from all active participants and collected from 32,826 NHS members in 1989–1990 and 18,225 HPFS members in 1993–1994.(29) With the exception of a modestly lower prevalence of smoking, those who returned blood samples did not differ substantially from those who did not in both cohorts, including average alcohol intake of 12.8 versus 12.2 grams/day among men and 6.5 versus 6.3 grams/day among women. Participants underwent local phlebotomy and returned samples to our laboratory via overnight courier. Upon arrival, whole blood samples were centrifuged and stored in cryotubes as plasma, buffy coat, and red blood cells in the vapor phase of liquid nitrogen freezers.

DNA was extracted from the buffy coat fraction of centrifuged blood with the QIAamp Blood Kit (Qiagen, Chatsworth, CA). The primary genotyping technique was Taqman SNP allelic discrimination by means of an ABI 7900HT (Applied Biosystems, Foster City, CA). The Q192R and L55M SNPs were genotyped with NCBI Reference SNP Cluster Identifiers rs662 and rs854560, respectively.

## Assessment of Diet and Smoking

We assessed diet with a 131-item semiquantitative food frequency questionnaire administered every four years that includes separate items for beer, white wine, red wine, and liquor, as previously described(30). We previously validated estimated alcohol consumption against two one-week dietary records collected approximately six months apart from 136 HPFS participants and 173 NHS participants residing in Eastern Massachusetts(31), with Spearman correlation coefficients between these two measures of 0.90 in women and 0.86 in men. In a similar validation study, the correlation coefficient between questionnaire and dietary-record estimates of energy-adjusted monounsaturated fat was 0.68.(32) Smoking has been assessed in cigarettes per day by questionnaire every two years.

In these analyses, we used estimates of alcohol, smoking, and energy-adjusted monounsaturated fat intake from 1990 among women and 1994 among men (i.e., at the time of the blood draw), using previous assessments when missing data occurred.

## Ascertainment of CHD

The outcome for this analysis was incident CHD, defined as non-fatal myocardial infarction or fatal CHD. We wrote to participants who reported incident CHD on the follow-up questionnaires to confirm the report and request permission to review medical records. We also sought medical records for deceased participants, whose deaths were identified by families and postal officials and through the National Death Index. Physicians blinded to the participant's questionnaire reports reviewed all medical records. Cases were identified primarily through review of medical records, as previously described.(28, 30)

## Case-Control Sampling

We performed parallel nested case-control studies within the samples of men and women who provided blood samples (Figure 1). In the NHS, we identified 249 women free of cardiovascular disease or cancer in 1990 who sustained an incident MI prior to June 30, 1998. In the HPFS, 266 men free of cardiovascular disease in 1994 developed incident CHD prior to January 31, 2000. For each incident case of CHD, we randomly selected two men or women free of cardiovascular disease matched on age (in five-year increments), smoking (in five categories), and month of blood return using risk-set sampling.(33) In the NHS, matching criteria also included fasting status and reported problems with blood drawing.

## Statistical Analysis

We calculated odds ratios from unconditional logistic regression models as measures of relative risk, including the matching variables in all analyses to approximate conditional logistic regression but more flexibly accommodate stratified analyses in which the members of matched pairs occupy different strata; primary results using conditional logistic regression were not appreciably different. In multivariate analyses of genotype, we additionally controlled for body mass index (in 5-kg/m<sup>2</sup> increments), diabetes, hypertension, hypercholesterolemia, average daily exertion (in five categories), aspirin use, and alcohol use. We also adjusted for current hormone replacement therapy use among women. We used covariate information from the time of the blood draw, defined as 1994 in the HPFS and 1990 in the NHS. We used information from previous questionnaires when covariate data from the time of blood draw were missing.

We tested main effects of genotype both as dominant and per-allele effects. To test interactions, we dichotomized lifestyle factors and examined genotypes within strata as dominant effects, given the limited number of variant homozygotes. We then tested multiplicative interactions (i.e., dominant genotype x dichotomized lifestyle exposure) in

models that included main effects and covariates. Based upon the distributions of alcohol intake and their relations with CHD in these cohorts,(34) we used cutpoints of 2.5 gm/day in women and 6 gm/day in men; examination of all drinkers versus abstainers or alcohol as a continuous variable tended to yield similar or weaker results that are not shown here. We dichotomized monounsaturated fat at the sex-specific median value and smoking as current versus former/never.

## Results

### Baseline Characteristics

Table 1 presents selected characteristics of NHS and HPFS participants according to case-control status. On average, the prevalence of cardiovascular risk factors was higher and alcohol intake lower among cases than controls, as expected.

Table 2 shows the distribution of PON1 genotypes among men and women. We found no departures from Hardy-Weinberg equilibrium for L55M or Q192R among either men or women.

### PON1 Polymorphisms and Risk of MI

Table 2 also shows the case-control status of men and women according to PON1 genotypes. Neither SNP was associated with risk in unadjusted analyses among either men or women. Likewise, neither SNP was significantly associated with risk in multivariable analyses in either sex, whether adjusted for matching factors or additionally for cardiovascular risk factors. In women, the multivariable-adjusted odds ratios were 1.11 (95% confidence interval [CI], 0.79–1.58) for Q192R and 0.98 (95% CI, 0.69–1.40) for L55M. The corresponding odds ratios among men were 1.02 (95% CI, 0.75–1.39) and 1.18 (95% CI, 0.85–1.63).

Given the effect of alcohol on PON1 activity, we next examined the risk for CHD associated with each SNP, stratified by alcohol consumption. Among women (Figure 2), the Q192R allele modelled dominantly tended to be associated with modestly higher risk for CHD among abstainers and rare drinkers, but not among consumers of 2.5 or more gm/day. There were similar but weaker trends for the L55M allele, with lower risk associated with the variant allele among abstainers and rare drinkers but higher risk among moderate drinkers. There was no evidence of interaction among men for either the Q192R ( $p=0.96$ ) or L55M ( $p=0.83$ ) alleles, even after exclusion of men consuming 50 or more gm/day (to provide greatest comparability to women).

To illustrate how these PON1 SNPs influence the association of alcohol intake with risk among women, we examined the association of alcohol intake of 2.5 gm/day or more with CHD risk among women with various genotypes. Alcohol intake of 2.5 gm/day or more was associated with lower risk among women with a Q192R allele (odds ratio 0.54; 95% CI, 0.32–0.92), but not among Q192 homozygotes (odds ratio 1.29; 95% CI, 0.74–2.27). Likewise, intake of 2.5 gm/day or more was associated with lower risk among L55 homozygotes (odds ratio 0.50; 95% CI, 0.27–0.91), but not among L55M carriers (odds ratio 1.12; 95% CI, 0.66–1.91). In jointly stratified analyses, the lower risk associated with alcohol intake was similar among all women (odds ratio 0.45; 95% CI, 0.29–0.70) except those with the Q192Q/L55M genotype (odds ratio 1.33; 95% CI, 0.73–2.40;  $p$  3-way interaction 0.07).

We also examined the interactions of monounsaturated fat intake and smoking with PON1 genotype on risk of CHD. Among women, tests for interaction were not significant for Q192R and monounsaturated fat ( $p=0.80$ ) or smoking ( $p=0.95$ ); the corresponding  $p$ -values

for L55M were 0.61 and 0.17, respectively. Likewise among men, there were no interactions of Q192R with monounsaturated fat intake ( $p=0.53$ ) or smoking ( $p=0.67$ ), or of L55M with either factor ( $p=0.49$  and  $0.61$ , respectively).

## Discussion

In this prospective study, two well-studied PON1 SNPs were not independently associated with risk of CHD. We found limited evidence that they may interact with alcohol intake to influence coronary risk, but only among women. Monounsaturated fat intake and smoking, other proposed modulators of PON activity, did not interact similarly with genotype.

Substantial evidence supports a link between alcohol use and PON activity. In two Dutch randomized crossover trials, intake of 30–40 gm of alcohol daily for three weeks (whether as beer, wine, or spirits) increased serum PON activity,(12, 13) although a smaller pre-post trial of red wine did not show an effect on PON activity.(35) Experiments with rats fed alcohol as 10% of calories also showed greater serum PON activity and greater hepatic PON mRNA expression; interestingly, this effect was actually reversed among rats fed alcohol as 36% of calories.(11) A small number of observational studies also support this link. In a study of 30 healthy individuals, Rao and colleagues found that consumers of 1–3 drinks per day had 4-fold greater PON activity than did non-drinkers, while consumers of 6 or more drinks per day had 45% lower activity, consistent with their findings in rats.(11) In a Chinese Han population, Wang and colleagues found that the alcohol intake was associated with greater PON activity, although the authors did not examine interaction with the Q192R polymorphism (which did not itself influence PON activity).(14) In two other observational studies, Ferre and colleagues did not find an association of alcohol intake with PON activity,(21) nor did investigators from the Stanislas Cohort Study,(22) although neither examined alcohol-genotype interactions.

We did not have information in this study on PON activity or other biochemical phenotypes that would internally support the observed interaction of alcohol and PON1 genotype. However, a recent large cross-sectional study found that the effect of alcohol on PON activity tended to be strongest among carriers of the 192R allele, directly consistent with our findings on CHD.(36) Together with structural evidence that the 192 position mediates HDL binding and hence PON1 stability,(37) it seems plausible that alcohol interacts with PON1 genotype by increasing levels of both HDL(10) and PON1 expression itself(11), effects that would be expected to increase PON activity (and hence reduce oxidative stress) predominately in carriers of the 192R allele who exhibit greater binding of PON1 to HDL and greater antioxidant activity.(7) Nonetheless, we cannot exclude the possibility of chance in the observed interaction, and we encourage further research into the interaction of alcohol with this and other possible genes involved in HDL metabolism.(38)

Although ample mechanisms exist to suggest that an inverse relationship between PON activity and risk of CHD is plausible, such a relationship has been difficult to identify to date. Despite the established effects of SNPs at positions 192 and 55 on PON activity in several studies,(39) meta-analyses suggest that these SNPs are not associated overall with risk of CHD.(3, 4) However, small studies published subsequent to these meta-analyses continue to suggest possible associations between PON1 SNPs and cardiovascular risk.(5–7, 40–46) Studies of directly measured PON activity are equally conflicting.(7, 47, 48) despite continued interest in the role of oxidative stress (which PON inhibits) in CHD.(49, 50) Given our results, the conflicting or null results of PON1 SNPs seen in previous studies may reflect the blending of differential effects among drinkers and non-drinkers in populations with varying distributions of alcohol consumption.

We did not find interactions of lifestyle factors other than alcohol with PON1 genotype. This specificity provides some support for the interaction with alcohol intake, but should not be taken as evidence that monounsaturated fat intake or smoking do not influence PON1 activity. Particularly strong evidence suggests that smoking decreases PON activity(51) and even that smoking cessation restores its activity.(52) Rather, our results merely suggest that these factors do not interact with the coding SNPs studied here and are not likely explanations for the heterogeneity in studies of PON1 and CHD.

Specific limitations and complexities of our study warrant discussion. We observed possible interactions of PON1 genotype and alcohol intake only among women. This may reflect a false-negative finding among men, particularly given the stronger relationship of alcohol consumption and risk of CHD among women than men in these cohorts.(34) Alternately, the apparent interactions among women may simply have occurred by chance, and indeed even the interactions terms between alcohol and genotype among women were not statistically significant by convention. However, sex steroid hormones themselves influence PON activity(53–55) and sex has been found to modify the association of PON1 variants with CHD in earlier studies.(45)

As in any observational study, our results could be influenced, at least in part, by differences between participants in factors other than alcohol consumption. In this study, we compiled extensive information on smoking, diet, exercise, body-mass index, aspirin use, and cardiovascular risk factors, and our populations are homogeneous with respect to occupational class and sex. Nonetheless, it is impossible to exclude the possibility that our results are confounded by lifestyle factors related to alcohol use, or to other SNPs in linkage disequilibrium with those evaluated here. Also, although this study is large and prospective, our results are necessarily limited by the population frequencies of individual alleles and the prevalence of alcohol consumption.

In summary, PON1 polymorphisms do not predict risk of coronary heart disease independently in these populations of men and women. Limited evidence suggests they may interact with alcohol intake to influence risk, but only among women. This gene-diet interaction should be evaluated in future studies of PON1 and coronary heart disease, and adds to a growing body of literature suggesting that genes modulate many of the diverse health effects of alcohol.

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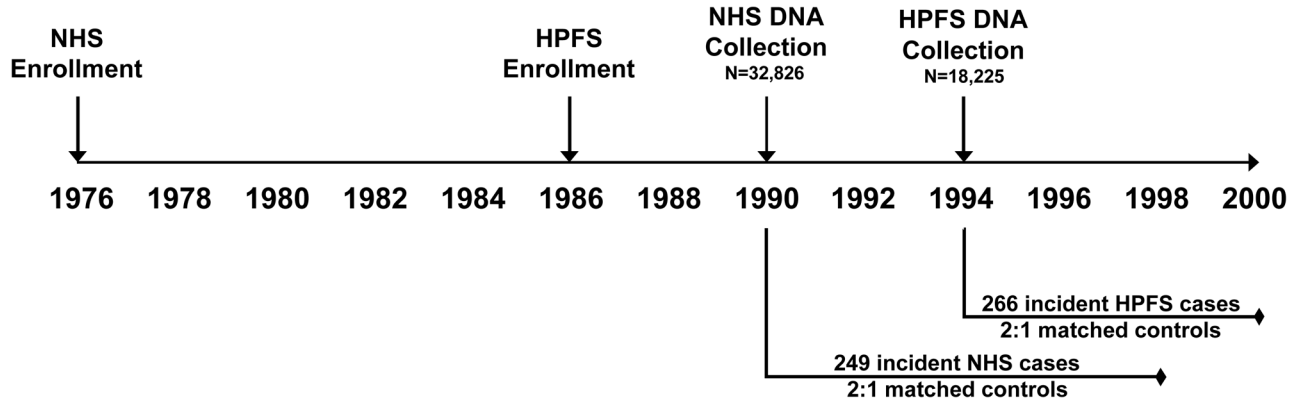
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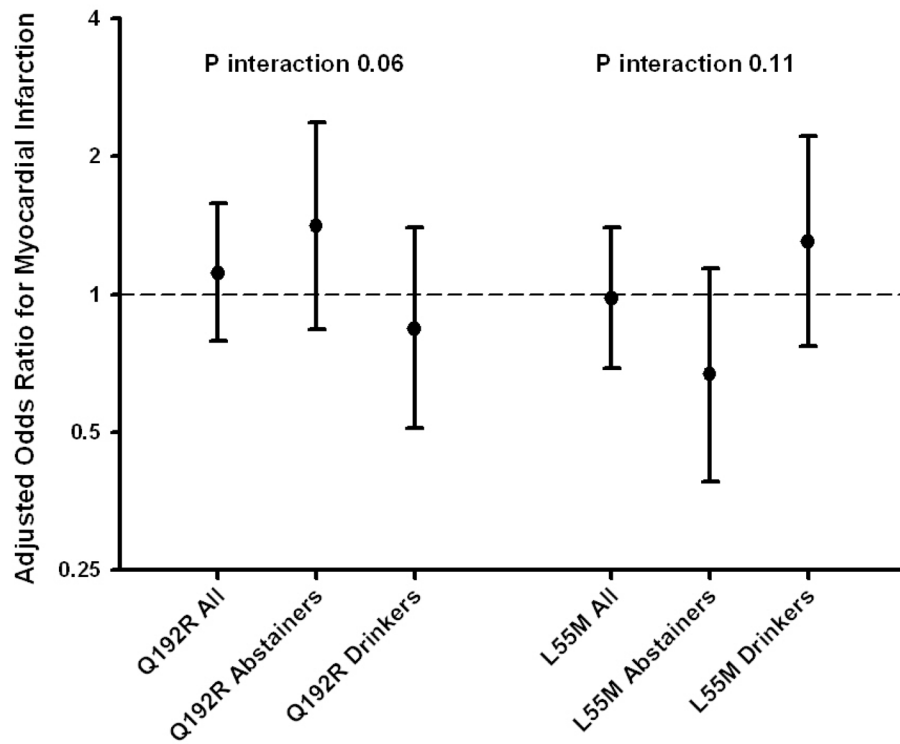


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**Figure 1.** Schematic representation of Nurses’ Health Study and Health Professionals Follow-up Study follow-up and case-control sampling. The NHS began in 1976 and collected blood specimens in 1990, from which genotyping was performed. The HPFS began in 1986, with blood collection in 1994. Among those who provided blood specimens, 249 cases of incident coronary heart disease in the NHS were identified by 1998, and 266 cases were identified in the HPFS by 2000. These cases were matched 2:1 with controls free of coronary heart disease at the time of case identification.



**Figure 2.**

Adjusted odds ratios for myocardial infarction associated with PON1 polymorphisms Q192R and L55M among women in the Nurses' Health Study.

T-bars indicate 95% confidence intervals. Odds ratios assume dominant main effects and were adjusted for case-control matching factors (age, smoking, month of blood draw, fasting status, problems with phlebotomy), body mass index, diabetes, hypertension, hypercholesterolemia, physical exertion, aspirin use, and hormone replacement therapy in logistic regression models. Abstainers include women consuming <2.5 gm/day of alcohol.

**Table 1**

Selected baseline characteristics according to case-control status.

Variable	Women		Men	
	Cases (n=243)	Controls (n=490)	Cases (n=263)	Controls (n=528)
Age (yrs)	60 ± 6	60 ± 6	65 ± 8	65 ± 8
Current smoker	33%	32%	12%	12%
BMI 25.0–29.9 kg/m <sup>2</sup>	29%	31%	51%	44%
BMI 30.0–34.9 kg/m <sup>2</sup>	16%	11%	9%	10%
BMI 35.0+ kg/m <sup>2</sup>	9%	3%	2%	1%
Diabetes	20%	7%	10%	5%
Hypertension	58%	29%	42%	31%
Hypercholesterolemia	54%	40%	49%	40%
Family history of MI	44%	28%	15%	11%
Postmenopausal hormone use	31%	36%		
Premenopausal	9%	12%		
Alcohol (g/d)	4 ± 9	6 ± 10	11 ± 16	13 ± 17
Dietary fiber (g/d)	19 ± 7	20 ± 8	23 ± 9	23 ± 9
-3 fatty acids (g/d)	0.25 ± 0.21	0.27 ± 0.36	0.28 ± 0.27	0.29 ± 0.43
Folate (µg/d)	453 ± 243	459 ± 253	515 ± 272	517 ± 276
Monounsaturated fatty acids (g/d)	24 ± 10	24 ± 10	28 ± 12	27 ± 13

Age and smoking were matched between cases and controls. Means and standard deviations are shown for continuous variables.

Table 2

Case-control status according to PON1 genotype.

	Total	HWE	Case	Control	P-value
<b>WOMEN</b>					
<b>L55M</b>					
LL	292		102 (44)	190 (41)	
LM	318	0.23	104 (45)	214 (46)	0.59
MM	89		26 (11)	63 (13)	
<b>Q192R</b>					
QQ	359		118 (51)	241 (52)	
QR	274	0.87	97 (42)	177 (38)	0.47
RR	65		18 (8)	47 (10)	
<b>MEN</b>					
<b>L55M</b>					
LL	315		96 (38)	219 (43)	
LM	335	0.21	116 (46)	219 (43)	0.38
MM	104		38 (15)	66 (13)	
<b>Q192R</b>					
QQ	403		131 (51)	272 (52)	
QR	301	0.32	104 (41)	197 (38)	0.62
RR	70		20 (8)	50 (10)	

Totals do not add to 747 in women or 798 in men because of missing genotypes.

P-values for the associations of genotype with case-control status derive from  $\chi^2$  tests. HWE indicates the p-value for Hardy-Weinberg Equilibrium.