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## Relationship of Human Paraoxonase-1 (PON1) Serum Activity and Genotype with Atherosclerosis in Individuals from the Deep South

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### Abstract

**OBJECTIVE**—Paraoxonase 1 (PON1) is synthesized in the liver and bound to high density lipoprotein (HDL) particles in blood. PON1 protects against the development of atherosclerosis by metabolizing pro-atherogenic oxidized lipids. The Southeastern United States (excluding Florida) has the country's highest age-adjusted mortality rate from cardiovascular disease. The current study determines the association of PON1 status with atherosclerosis (ATH) in individuals from the Southeastern United States.

**METHODS**—Eighty African Americans (40 male, 40 female) and 120 Caucasians (60 male, 60 female) were enrolled from a cardiology practice in northeastern Mississippi. Serum PON1 activities were determined using diazoxon, paraoxon, and phenyl acetate (PhAc) as substrates. The *PON1*<sub>192</sub> genotype of each individual was also determined. A multivariable logistic regression model was developed to identify the associations of clinical characteristics, serum PON1 activity, and *PON1*<sub>192</sub> genotype of the study population with ATH.

**RESULTS**—A core model consisting of age, gender, history of smoking, hypertension, and LDL-cholesterol group was constructed. The maximum-rescaled generalized  $r^2$  value for the core model

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The authors declare they have no competing financial interests.

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was 0.35. Addition of PON1 activity assessed by PhAc hydrolysis was the only measure of PON1 enzymatic activity to add significant information to the core model ( $p=0.0317$ ) with the maximum-rescaled generalized  $r^2$  value increasing to 0.37. Increasing PON1 activity was associated with decreased odds of ATH. The *PON1*<sub>192</sub> genotype was not significantly associated with ATH.

**CONCLUSIONS**—Increasing PON1 activity assessed by the hydrolysis of PhAc is associated with decreased odds of ATH in a group of African American and Caucasian Southerners.

### Keywords

paraoxonase; atherosclerosis; coronary artery disease; cardiovascular disease; health disparities

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## INTRODUCTION

The paraoxonase (PON) gene family has been extensively investigated recently. One member of the PON family, paraoxonase-1 (PON1) has been implicated in a wide variety of human illnesses including cardiovascular disease (CVD), diabetes mellitus, and obesity [1-3]. PON1 performs multiple functions. In humans, PON1 is mainly expressed in the liver, then transported to the blood where it is tightly bound to high density lipoprotein (HDL) particles ( $K_D < 1$  nM) [4]. One physiological function is the prevention of low density lipoprotein (LDL) and HDL oxidation by hydrolyzing lipid peroxides in lipoprotein particles; therefore it may protect against the development of atherosclerosis (ATH) [3, 5]. PON1 also protects against the acute toxicity of some organophosphorus anticholinesterase insecticides through hydrolysis of the compounds or their active metabolites [6]. In fact, paraoxonase received its name for its ability to hydrolyze paraoxon, the active metabolite of the insecticide parathion.

Studies using mouse models of ATH (ApoE<sup>-/-</sup> and LDL-R<sup>-/-</sup>) have consistently shown that the over expression of PON1 reduces ATH by reducing oxidative stress, hydrolyzing lipid peroxides, promoting cholesterol efflux from macrophages, and increasing reverse cholesterol transport [7-9]. Conversely, studies have shown that PON1-deficient mice have increased susceptibility to LDL oxidation, increased measures of macrophage oxidative stress, and larger atherosclerotic lesions than controls [10, 11].

Differences in PON1 activity have been linked to common polymorphisms in both coding and promoter regions [12, 13]. Two common polymorphisms in the coding region of PON1 are PON1 L55M and PON1 Q192R. The PON1 Q192R polymorphism has the greater influence on enzyme activity measured *in vitro* [12, 14]. This polymorphism involves a glutamine (Q) to arginine (R) substitution at position 192 [14, 15]. Several promoter region polymorphisms have been described with the C-108T polymorphism influencing expression the most [13]. The recognition that PON1 prevents lipid peroxide accumulation and that the PON1 Q192R polymorphism affects the rate of hydrolysis of several pesticide metabolites led to investigation of the possible association of the PON1 Q192R polymorphism with atherosclerosis.

The relationship, if any, of the PON1 Q192R polymorphism with the development of atherosclerosis is unclear. Early research suggested that the PON1<sub>192Q</sub> alloform was more efficient at metabolizing lipid peroxides in human atherosclerotic plaques than the PON1<sub>192R</sub> alloform [16], and thus was believed to be more effective at preventing ATH. In addition several early studies (reviewed in Costa et al. [17]) as well as two more recent reports [18, 19] have suggested an association of the *PON1*<sub>192R</sub> allele with atherosclerosis. Other studies have indicated that the PON1<sub>192R</sub> alloform binds to HDL with greater affinity than the PON1<sub>192Q</sub> alloform and therefore demonstrates increased stability and greater lipolactonase activity [20]. Also the prevalence of ATH was greatest in subjects with the *PON1*<sub>192QQ</sub> genotype [21]. A recent meta-analysis found only a weak association of *PON1*<sub>192R</sub> with vascular disease and pointed out the need for more definitive studies [22]. Richter and Furlong [23] have argued that to accurately assess PON1's role in atherosclerosis that both PON1 genotype and phenotype (i.e. activity level), together known as the PON1 status, must be determined.

An individual's functional *PON1*<sub>192</sub> genotype can be determined using a 2-substrate assay to generate a 2-dimensional enzyme activity plot [23]. Without actual genotyping, the plot of the rates of diazoxon hydrolysis in the presence of 2 M NaCl (diazoxonase; DZOaseNa) vs. paraoxon hydrolysis in the presence of 2 M NaCl (paraoxonase; POaseNa) separates individuals into 3 functional genotypes, PON1<sub>192QQ</sub>, PON1<sub>192QR</sub> and PON1<sub>192RR</sub>. PON1 activity determined using PhAc as a substrate in the absence of NaCl does not distinguish between the PON1<sub>192Q</sub> and the PON1<sub>192R</sub> alloforms and thus serves as a measure of PON1 activity independent of genotype [24].

Numerous epidemiological studies have identified factors that increase the risk of ATH. Important risk factors include: dyslipidemias (e.g., low HDL cholesterol and high LDL cholesterol), smoking, diabetes mellitus, and hypertension. States in the Southeast (except Florida) have higher age-adjusted annual mortality rates from CVD than any other geographic region in the US [25]. The cause for this disparity is unknown. Low socioeconomic status, exposure to environmental toxicants, and the increasing prevalence of hypertension have been proposed to explain the higher stroke mortality rate in the South [26]. African American men and women have higher age-adjusted mortality rates from CVD than Caucasian men and women [25]. Little information is available on the frequency distribution of *PON1*<sub>192</sub> genotypes of US Southern populations, which have among the worst health statistics in America. Early studies reported that African Americans have a weighted *PON1*<sub>192Q</sub> allele frequency of 0.37 and a weighted *PON1*<sub>192R</sub> allele frequency of 0.63, while Caucasians have a weighted *PON1*<sub>192Q</sub> allele frequency of 0.73 and a weighted *PON1*<sub>192R</sub> allele frequency of 0.27 [27]. A study from our laboratory [28] showed a similar distribution with African Americans having a higher *PON1*<sub>192R</sub> allele frequency, 0.66, and Caucasians having a higher *PON1*<sub>192Q</sub> allele frequency, 0.77.

In the present study, the *PON1*<sub>192</sub> genotype and the PON1 enzymatic activity (phenotype) were determined for African American and Caucasian male and female Southerners for whom demographic and clinical information was available. The enzymatic activity was assessed with three substrates, paraoxon, diazoxon and PhAc. Statistically significant associations of clinical characteristics and *PON1*<sub>192</sub> genotype and phenotype with ATH were

determined using logistic regression. A model was developed to test the association of PON1 status with ATH while accounting for a core group of clinical and demographic variables.

## MATERIALS AND METHODS

### Study Population

Subjects were enrolled in the study from the clinical practice of Cardiology Associates of North Mississippi, LLC, a cardiology group located in Tupelo, Mississippi, which draws the vast majority of its patients from the 26 counties comprising northeast Mississippi and northwest Alabama. The research division of this clinic, Cardiology Associates Research, LLC (CARE), collected a total of 200 serum samples from Caucasians (60 male, 60 female) and African Americans (40 male, 40 female), ages 45 and older. A 60/40 split was chosen to reflect the racial composition of Mississippi.

Subjects were excluded if they were being evaluated for acute coronary syndrome (recent myocardial infarction or unstable angina), were known or suspected to be infected with human immunodeficiency virus or hepatitis B, or did not self-declare as either Caucasian or African American. Participants underwent the diagnostic tests recommended by their cardiologist. Study records that identified the subject were kept confidential as required by Federal Privacy Regulations. The protocol was approved by both the Institutional Review Board (IRB) of Mississippi State University and the IRB of North Mississippi Medical Center; the latter provides oversight for studies performed at CARE.

After obtaining informed written consent, blood samples were collected when vascular access was established for clinical testing. Serum was isolated by allowing blood to clot, then centrifuging at  $2500\text{--}3000 \times g$  at  $4^{\circ}\text{C}$  for 10 to 20 minutes. The serum was frozen on dry ice and stored at  $-70^{\circ}\text{C}$  until analysis. An aliquot of whole blood anti-coagulated with  $\text{K}_2\text{EDTA}$  was stored at  $-70^{\circ}\text{C}$  for *PON1* genotypic analysis.

Age, gender, race, diabetes, history of tobacco use, family history of coronary artery disease or stroke, presence of hypertension, the use of statins, height, weight, waist circumference in inches, blood pressure, and pulse were also obtained for each individual. A fasting lipid panel was obtained on the serum collected. All clinical information was de-identified before release from the clinic. Subjects were classified as having ATH if they had either a history of a clinical event resulting from or a mechanical revascularization procedure for treating coronary artery disease, cerebrovascular disease, abdomino-aortic disease, or peripheral vascular disease. Subjects were also considered to have ATH if they had reversible perfusion defects interpreted as myocardial ischemia on a nuclear stress test. Subjects who did not meet the criteria for ATH were considered not to have ATH. All diagnostic tests were interpreted by a CARE physician who had no knowledge of the results of the PON1 assays.

Statin usage of a subject was designated as 'yes' if the subject was currently on a statin drug or designated as 'no' if the subject was not taking a statin. Smoking status of an individual was assigned an 'ever' if the subject was still smoking or had ever smoked or 'never' if the subject had never smoked.

Because of the large number of participants on statins, LDL cholesterol levels and statin usage were used to place subjects into one of two categories. Subjects were considered normal for LDL cholesterol if their levels were  $\leq 160$  mg/dL, they did not meet criteria for pharmacologic treatment [29], and they were not currently taking a statin. Subjects' LDL cholesterol levels were considered elevated if their levels were  $>160$  mg/dL, they were currently taking a statin, or they met the criteria for pharmacological treatment of elevated LDL cholesterol [29]. Subjects were considered normal for HDL cholesterol if their levels were  $\geq 40$  mg/dL and low if their levels were  $< 40$  mg/dL.

Subjects were considered normal for triglycerides if their levels were  $<200$  mg/dL and they were not taking a fibrate. Subjects were considered abnormal for triglycerides if their levels were  $\geq 200$  mg/dL or they were taking a fibrate. Subjects who were on statin therapy and had triglycerides  $<200$  mg/dL were considered indeterminate as it was assumed statin therapy was prescribed for elevated LDL cholesterol but would influence triglyceride levels and prevent an accurate designation of baseline triglyceride status (i.e., off statin therapy).

Subjects were considered normal for total cholesterol if their levels were  $<200$  mg/dL and they were not taking statins. Subjects were considered abnormal for total cholesterol if their levels were  $\geq 200$  mg/dL. Subjects who were on statin therapy and had a total cholesterol  $<200$  mg/dL were considered indeterminate as it was assumed statin therapy was prescribed for elevated LDL cholesterol but would influence total cholesterol levels and prevent an accurate designation of baseline total cholesterol status (i.e., off statin therapy).

### Chemicals and Samples

Paraoxon was synthesized as described previously [30]. Diazoxon was purchased from ChemService (West Chester, PA). All other chemicals were purchased from Sigma Chemical Company (St. Louis, MO).

### Paraoxonase and Diazoxonase Assays

Paraoxonase and diazoxonase activities were determined by the hydrolysis of paraoxon and diazoxon, respectively, as described by Richter and Furlong [23] with minor modifications [28]. The paraoxonase assay was conducted in the presence of 2 M NaCl (POaseNa) and the absence of NaCl (POase). The diazoxonase assay was conducted in the presence of 2 M NaCl (DZOaseNa) and the absence of NaCl (DZOase). Samples were run in triplicate. Data were expressed as units per liter of serum (U/L) where 1 unit = 1 micromole of paraoxon or diazoxon hydrolyzed per minute.

The functional genotype was determined by plotting the DZOaseNa activity versus the POaseNa activity for each sample [23].

### Arylesterase Assay

Arylesterase activity was determined using PhAc as a substrate. The phenol released from PhAc hydrolysis was measured spectrophotometrically as described previously [28] and the activity was expressed as U/L where 1 unit = 1 micromole of PhAc hydrolyzed per minute. Samples were run in triplicate.

### PON1 Genotyping

Genomic DNA was isolated from whole blood using a Sigma-Aldrich® GenElute™ Blood Genomic DNA Kit and concentrated using a Qiagen QIAEX®II Gel Extraction Kit. Following the method received from the Medical Genetics Department of the University of Washington (Jane Ranchalis, personal communication), a region containing the *PON1*<sub>192</sub> codon was amplified by PCR to yield a 99 bp product using the primer pair F 5' TATTGTTGCTGTGGGACCTGAG 3' and R 5' TGAAAGACTTAAACT 3'. Each PCR reaction contained ~200 ng of purified human DNA at ~100 ng/μl, 2 μl of 10X AmpliTaq Gold® 360 PCR Buffer (ABI), 2 μl of 25 mM MgCl<sub>2</sub>, 0.16 μl 25 mM dNTPs (Promega), 0.2 μl of each primer at 25 μM, 0.2 μl AmpliTaq Gold polymerase (ABI) at 5U/μl, 1 μl of dimethylsulfoxide and water sufficient to bring the final volume to 20 μl. Since there is a native *A/w*I restriction site at codon 192, 10 μl of the PCR product solution was digested with *A/w*I (NEB) overnight and visualized by ethidium bromide staining on a 3% agarose gel next to 10 μl of undigested product solution. The digestion resulted in 63- and 36-bp fragments for individuals with the *PON1*<sub>192RR</sub> genotype; 99-, 63-, and 36-bp fragments for individuals with the *PON1*<sub>192QR</sub> genotype; and a single, non-digested 99-bp fragment for individuals with the *PON1*<sub>192QQ</sub> genotype. Sequence data confirmed the results from the restriction digest analyses for 50 of the 200 participants sequenced.

### Statistical Analysis and Model Development

SAS for Windows 9.1.3 (SAS Institute Inc., Cary, NC, USA) was used for statistical analysis. A chi-square ( $\chi^2$ ) test (PROC FREQ) with Bonferroni correction for multiple comparisons when a significant  $\chi^2$  was found ( $p < 0.05$ ), was used to determine differences among race-gender groups for PON1 enzymatic activities and genotype. One-way analysis of variance was used (PROC GLM) to determine if significant differences among race-gender groups ( $p < 0.05$ ) existed for continuous clinical variables. If the model indicated significant differences among groups, mean separation tests were conducted using the Tukey adjustment. Differences in mean POase activity, DZOase activity, and PhAc hydrolysis between subjects with or without atherosclerosis were assessed by t-test using PROC TTEST.

The strength of association between the occurrence of ATH and each of the explanatory variables listed in Table 3 was assessed by odds ratios using logistic regression (PROC LOGISTIC, SAS for Windows v. 9.1.3, SAS Institute, Cary, NC). Clinical variables that were found to be associated with ATH with a p-value  $< 0.25$  were used as candidates in a multivariable model [31]. After the model was fit, the variable with the highest p-value was removed manually and the remaining explanatory variables reassessed. This process was continued until only clinical variables with p-value  $< 0.05$  remained in the model. This multivariable model was designated the core factor model. The experimental variables for PON1 activity (i.e., activities for hydrolysis of paraoxon, diazoxon, and PhAc) were then added individually to the core factor model to test the association between ATH and the experimental variables for PON1 activity while accounting for the core clinical variables.

## RESULTS

### Characterization of the Study Population

Clinical variables were expressed as mean  $\pm$  SD for continuous data and ratios for categorical data for each race-gender group (Table 1). Height and weight were used to determine body mass index (BMI). The categorical variables LDL group, triglyceride (trig) group, and TotalChol group were determined using the appropriate values from the lipid panel and information of statin use as described in detail in the Methods section.

### PON1 Genotypes

Separation of the 200 individuals of this study into the three functional PON1<sub>192</sub> genotypes through the 2-dimensional plot of POaseNa vs. DZOaseNa was not as clear as previously reported [23, 28] (Figure 1a). In fact, the DZOaseNa vs POaseNa plot alone was unable to unambiguously assign a functional genotype to a number of individuals. Therefore, the *PON1*<sub>192</sub> genotype was determined using a restriction fragment length polymorphism assay. Assignment of genotypes to the data points on the DZOaseNa vs POaseNa plot showed that individuals with each of the three *PON1*<sub>192</sub> genotypes were located in the expected region of the plot (Figure 2). A greater portion of Caucasians had the *PON1*<sub>192QQ</sub> genotype (p = 0.0001) than did the African Americans, while the reverse was true for the *PON1*<sub>192RR</sub> genotype (p = 0.0001) (Figure 1b, c and Table 2). No significant differences were noted in the population distribution of the *PON1*<sub>192QR</sub> genotype (Table 2).

### Differences in Serum Enzyme Activities

Serum variables were expressed as mean  $\pm$  SD for each race-gender group (Table 2). African American females had significantly higher POaseNa activities than Caucasian females (p= 0.0005) and Caucasian males (p= 0.0002) but no differences between the other groups were observed (p> 0.1171). Caucasian females had significantly higher DZOaseNa activities than African American males (p= 0.0321) but no differences between the other groups were observed (p> 0.1470). African American females had significantly higher POase activities than Caucasian females (p= 0.0004) and Caucasian males (p= 0.0003) but no differences between the other groups were observed (p> 0.1382). No significant differences (p> 0.1054) were detected with DZOase activity levels among the race-gender groups. African American females had significantly higher PhAc activities than Caucasian females (p= 0.0216) and Caucasian males (p= 0.0049) but no difference between the other groups were observed (p> 0.2898).

### Associations of ATH with Clinical and Serum Factors

Statistically significant associations (p = 0.05) between the presence of ATH and each of the clinical and serum factors individually were determined by logistic regression (Table 3). In our study population, increasing age, gender, smoking status, and classification in the elevated LDL, the low HDL, and the abnormal triglyceride groups were all associated with ATH. Classification in the elevated LDL cholesterol group had the strongest association with ATH of all the explanatory variables. Surprisingly, increasing body mass index (BMI) was associated with decreased odds of ATH. Increased levels of PON1 activity (expressed as

POaseNa, DZOaseNa, POase, DZOase or PON1 activity determined by the hydrolysis of PhAc) were associated with decreased odds of ATH. Not surprisingly, individuals with ATH also had significantly lower PON1 activities than individuals without ATH using either paraoxon ( $89 \pm 51$  U/L vs.  $105 \pm 57$  U/L,  $p = 0.0316$ ) diazoxon ( $6033 \pm 2031$  U/L vs.  $6806 \pm 2382$  U/L,  $p = 0.0151$ ), or PhAc ( $14778 \pm 3163$  vs.  $15976 \pm 3430$ ,  $p = 0.0114$ ) as the substrate.

Factors that were included in the analysis but did not have statistically significant associations with ATH included race ( $p = 0.3264$ ), hypertension ( $p = 0.0898$ ), diabetes ( $p = 0.6778$ ), family history of ATH ( $p = 0.1242$ ), waist circumference ( $p = 0.8021$ ), total cholesterol group ( $p = 0.8427$ ), ratio LDL/HDL ( $p = 0.3466$ ), and *PON1*<sub>192</sub> genotype ( $p = 0.9792$ ).

### Multivariable Logistic Regression Model

A multivariable logistic regression model was developed that showed the association of individual clinical and serum factors with the occurrence of ATH (Table 4). Triglyceride and total cholesterol groups were not included as variables in the model because over half the sample population was indeterminate for the variables due to statin therapy. Statin usage was not included as a variable in the model because it was used to define the LDL cholesterol groups. The factors age, gender, smoking status, hypertension, and LDL cholesterol group remained in the model to constitute the core model. Age showed a direct association with ATH (OR=1.04,  $p = 0.0251$ ). Gender was associated with ATH (OR=3.13,  $p = 0.0011$ ), with males being more likely than females to have ATH. Subjects who had a history of smoking (OR=1.98,  $p = 0.0560$ ) or had hypertension (OR=2.05,  $p = 0.0888$ ) were more likely to have ATH. History of smoking and hypertension were both left in the core model, even though their  $p$ -values were  $>0.05$ , because they are well-established risk factors for ATH.

Following construction of the core factor model, the various PON1 activity measurements were added individually to the model to test the association of PON1 activity and ATH. PON1 activity as measured by PhAc hydrolysis was the only activity measurement that was significantly associated with ATH ( $p = 0.0317$ ). The presence of hydrolysis of PhAc in the model increased the maximum-rescaled generalized  $r^2$  value from 0.35 to 0.37.

## DISCUSSION

PON1 is now known to hydrolyze many substrates including organophosphorus insecticide metabolites and pharmaceuticals [6, 32]. PON1 has also been found to play an important role in the prevention of ATH by the metabolism of pro-atherogenic oxidized lipids [3]. To accurately assess the association of PON1 with ATH an individual's PON1 status (genotype and phenotype) must be determined. Even though individuals from the Deep South (except Floridians) have higher age-adjusted mortality rates of CVD than people in other regions of the United States, little is known about the PON1 status in the Southern population.

In the present study, each individual's PON1 status was determined. This analysis included determination of PON1 activity using the three substrates diazoxon, paraoxon, and PhAc and determination of each individual's *PON1*<sub>192</sub> genotype. A core model consisting of risk



factors known to be associated with ATH including age, gender, history of smoking, presence of hypertension, and LDL cholesterol group was developed. Family history was not associated with ATH in our study population possibly because many subjects were unable to provide enough information to accurately assign their family history. Diabetes also was not associated with ATH in our study population. The lack of association of diabetes with ATH may be the result of the over representation of diabetics in the group of individuals without ATH (35 of 104, more than would be expected in a random sampling of the population, 12.3 % prevalence of diabetes in MS). Most of the study subjects had been referred for diagnostic testing for coronary disease and, not surprisingly, had a much higher prevalence of diabetes than the general population. Increasing BMI was associated with decreased odds of ATH in our study population. The reason for this is not known though individuals with ATH may have undergone more intensive dietary counseling and been more motivated to follow the dietary recommendations than individuals without ATH.

The association of the various components of PON1 status with ATH was determined. Interestingly, all of the PON1 enzymatic measurements had an association with ATH when assessed individually. However, when assessed in the presence of the clinical factors comprising the core model, only PON1 activity determined by the hydrolysis of PhAc was associated with ATH with a  $p < 0.05$ . The maximum-rescaled generalized  $r^2$  value of the core model was 0.35. The addition of PON1 activity (assessed by the hydrolysis of PhAc) to the core model resulted in an increase of the maximum-rescaled generalized  $r^2$  value to 0.37. While this increase is not large, this small increase is of interest because only 5-10% of the risk of ATH is not accounted for by known risk factors in studies using very large numbers of patients [33]. Gupta et al. [18] have also reported an association of PON1 activity assessed by PhAc hydrolysis (as well as several other PON1 related characteristics) with atherosclerosis in Indian Punjabis.

The results of our study must be interpreted with a degree of caution. First, the study population was not randomly selected but consisted of individuals being treated or evaluated in a cardiology clinic. This selection bias resulted in an over representation of diabetes in individuals without ATH in the study population and prevented an adequate evaluation of diabetes in this study. Second, most patients with ATH in this study had a previous history of ATH and were already being treated (as evidenced by the extensive use of statins in the study population) thus allowing for the possible alteration of PON1 enzymatic activities. However, use of statins and fibrates has in general been associated with an increase in PON1 serum activity measures (reviewed in Paragh et al. [34]). If such an increase had occurred in the study population, it would obscure a sparing effect of PON1 activity on ATH occurrence. The ongoing statin treatment also resulted in the inability to include triglyceride levels and total cholesterol levels in the model. In addition, the only screening test for ATH was cardiac nuclear perfusion imaging for hemodynamically significant coronary artery disease. This may not have detected subclinical ATH as sensitively as some other imaging modalities used to diagnose atherosclerosis (e.g. carotid ultrasound) used in previously published studies [35, 36]. This would result in individuals with subclinical coronary artery disease being classified as normal i.e. no atherosclerosis. Despite these limitations, this study did demonstrate that increased PON1 activity, as measured by PhAc hydrolysis, is associated with decreased odds

of ATH in this Southern population. Interestingly, in this population the *PON1*<sub>192</sub> genotype was not significantly associated with ATH.

## CONCLUSIONS

This study documents a sparing effect of serum PON1 activity as assessed by the hydrolysis of PhAc on ATH occurrence adjusted for the effect of age, gender, history of smoking, hypertension, and elevated LDL-cholesterol. Diabetes, family history of ATH, total cholesterol levels and triglyceride levels are not well investigated in this model. The possibility of PON1 activity as an independent risk marker of ATH warrants further investigation to clarify these issues. This is the first study to document a relationship of PON1 activity with ATH in a population from the Deep South. In addition, the study has produced a model that will have utility for characterizing ATH risk factors utilizing larger data sets in the future.

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## ABBREVIATIONS AND DEFINITIONS

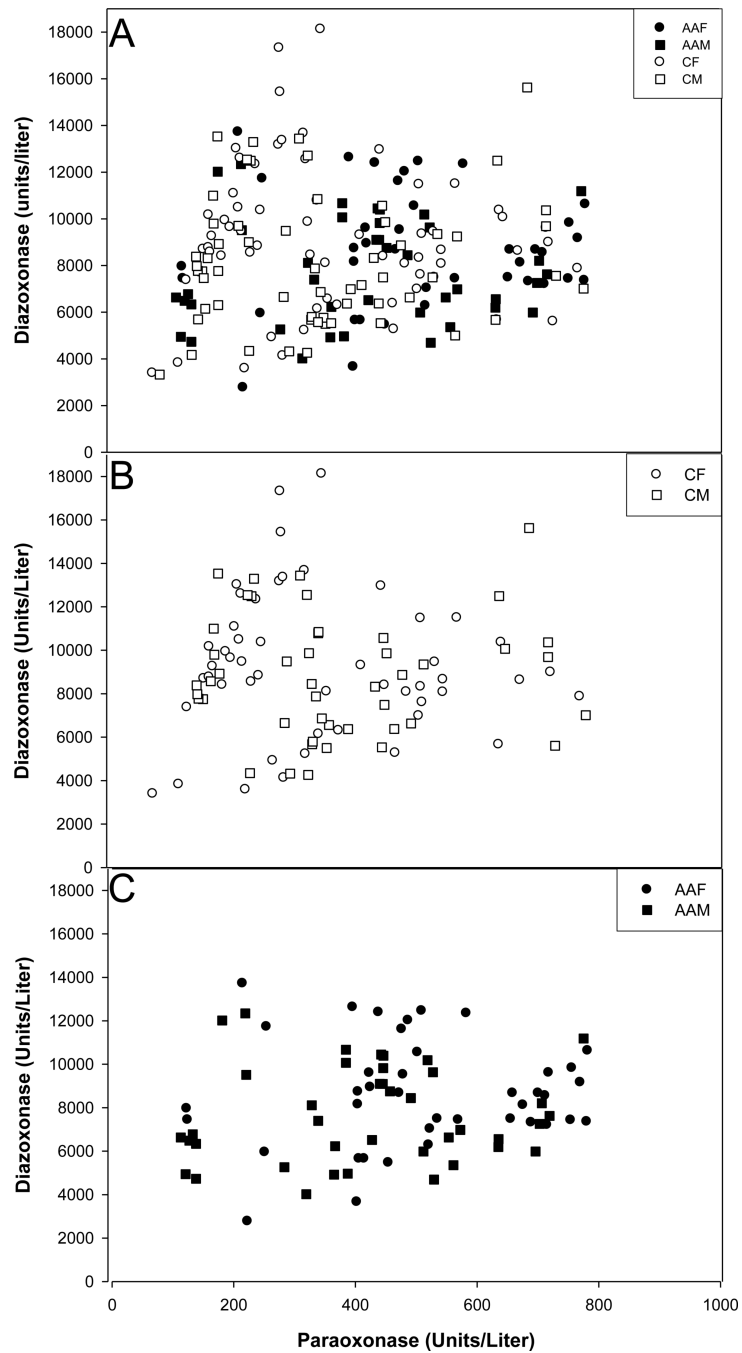
<b>ATH</b>	Atherosclerosis
<b>CVD</b>	cardiovascular disease
<b>DZOase</b>	diazoxonase, i.e., enzymatic activity of paraoxonase assessed with diazoxon with no NaCl present
<b>DZOaseNa</b>	diazoxonase, i.e., enzymatic activity of paraoxonase assessed with diazoxon with 2 M NaCl
<b>HDL</b>	high-density lipoprotein
<b>LDL</b>	low-density lipoprotein
<b>POase</b>	paraoxonase, i.e., enzymatic activity of paraoxonase assessed with paraoxon with no NaCl present
<b>POaseNa</b>	paraoxonase, i.e., enzymatic activity of paraoxonase assessed with paraoxon with 2 M NaCl
<b>PON1</b>	paraoxonase-1
<b>PhAc</b>	phenyl acetate

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**Figure 1. Plot of serum DZOaseNa vs. POaseNa enzymatic activities**

The sample population (n=200) consisted of 120 Caucasians (60 male, 60 female) and 80 African Americans (40 male, 40 female). (A) Plot of DZOaseNa activities vs. POaseNa activities in the sera of male and female African American and Caucasian Southerners. (B) Plot of DZOaseNa activities vs. POaseNa activities in the sera of male and female Caucasian Southerners. (C) Plot of DZOaseNa activities vs. POaseNa activities in the sera of male and female African American Southerners

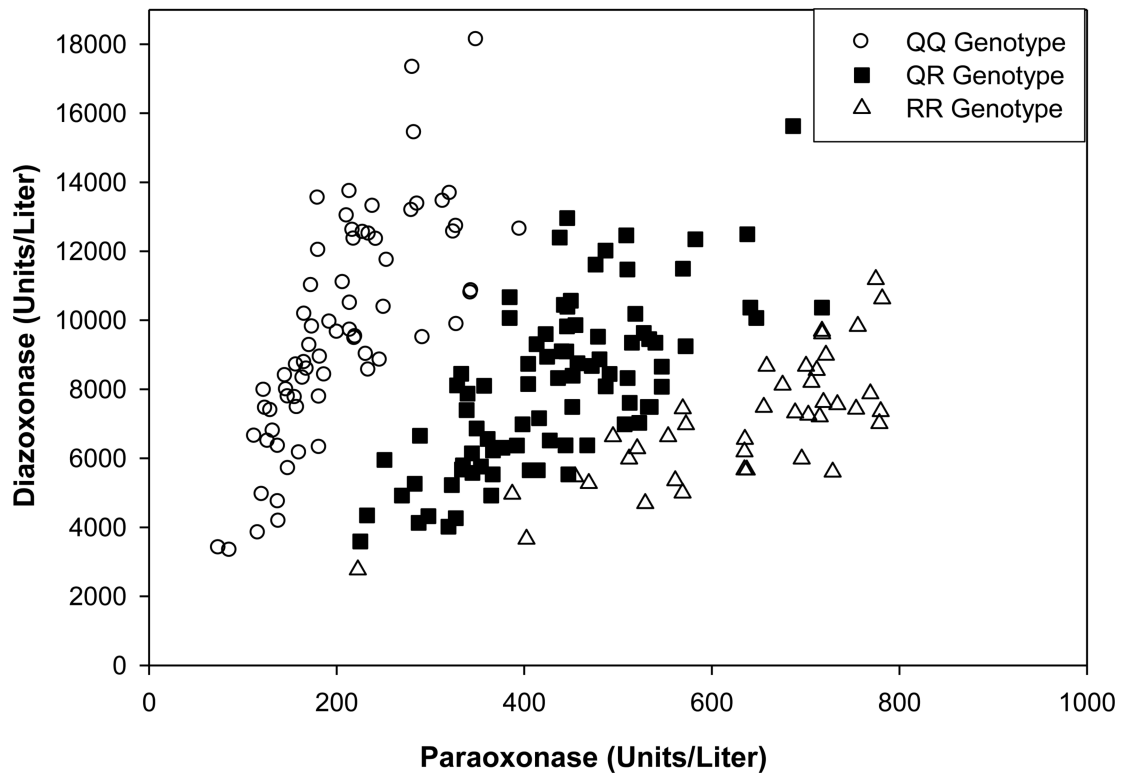
Abbreviations: AAF, African American Female (●); AAM, African American Male (■);  
CF, Caucasian Female (○); CM, Caucasian Male (□)

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**Figure 2. Plot of DZOaseNa vs. POaseNa enzymatic activities in the sera of male and female African American and Caucasian Southerners**

Genotypes are *PON1*<sub>192QQ</sub> (○); *PON1*<sub>192QR</sub> (■); and *PON1*<sub>192RR</sub> (△). The sample population (n=200) consisted of 120 Caucasians (60 male, 60 female) and 80 African Americans (40 male, 40 female)

**Table 1**

Characterization of study population by race-gender group.

Variables <sup>1</sup>	Units	TotalPop	AAF <sup>2</sup>	AAM <sup>2</sup>	CF <sup>2</sup>	CM <sup>2</sup>
		n=200	n=40	n=40	n=60	n=60
Age	Years	63 ± 9	60 ± 11	60 ± 7	65 ± 9	64 ± 9
Smoker (Ever/Never)		113/87	13/27	27/13	31/29	42/18
Diabetes (Yes/No)		70/130	18/22	15/25	19/41	18/42
Hypertension (Yes/No)		154/46	35/5	34/6	44/16	41/19
Height	Inches	67 ± 4	65 ± 3	69 ± 3	65 ± 3	70 ± 3
Weight	pounds	198 ± 44	197 ± 54	201 ± 36	183 ± 43	214 ± 38
BMI	kg/m <sup>2</sup>	31 ± 6	33 ± 8	30 ± 5	31 ± 7	30 ± 5
HDL	mg/dL	48 ± 16	56 ± 13	43 ± 12	51 ± 16	40 ± 13
LDL	mg/dL	107 ± 36	123 ± 37	98 ± 30	116 ± 40	95 ± 29
Triglycerides	mg/dL	149 ± 97	119 ± 53	126 ± 113	162 ± 73	171 ± 122
TotalChol	mg/dL	185 ± 45	203 ± 43	165 ± 34	202 ± 48	168 ± 39
Statin Use (Yes/No)		121/79	19/21	29/11	30/30	43/17
HDL group (Low/Normal)		76/120	5/34	18/22	14/44	39/20
LDL group (Elevated/Normal)		156/41	25/14	35/5	43/15	53/7
Trig group (Abnormal/Normal) <sup>3</sup>		43/63	3/19	5/9	19/20	16/15
TotalChol group (Abnormal/Normal) <sup>3</sup>		69/37	21/9	7/8	29/10	12/10
Atherosclerosis (Yes/No)		96/104	10/30	25/15	21/39	40/20

<sup>1</sup>Variables: Age, Height, Weight, Body Mass Index (BMI), Total Cholesterol, HDL cholesterol, and LDL cholesterol are expressed as means ± SD. Variables: Smoker, Diabetes, Hypertension, Statin Use, HDL group, LDL group, Trig group, and TotalChol group were expressed as ratios.

<sup>2</sup>AAF=African American Female, AAM=African American Male, CF=Caucasian Female, and CM=Caucasian Male.

<sup>3</sup>Subjects receiving statin therapy were not included in the summary statistics for these variables



**Table 2**

Serum PON1 parameters of Caucasian and African American males and females.

Variables	Units	TotalPop n = 200	AAF n = 40	AAM n = 40	CF n = 60	CM n = 60
POaseNa <sup>1</sup>	U/L	401 ± 188	509 ± 183 <sup>A</sup>	421 ± 189 <sup>AB</sup>	363 ± 173 <sup>B</sup>	354 ± 178 <sup>B</sup>
DZOaseNa <sup>1</sup>	U/L	8460 ± 2757	8649 ± 2566 <sup>AB</sup>	7699 ± 2231 <sup>B</sup>	9191 ± 3110 <sup>A</sup>	8130 ± 2741 <sup>AB</sup>
POase <sup>1</sup>	U/L	98 ± 54	129 ± 56 <sup>A</sup>	104 ± 53 <sup>AB</sup>	85 ± 50 <sup>B</sup>	85 ± 50 <sup>B</sup>
DZOase <sup>1</sup>	U/L	6437 ± 2249	6141 ± 1814 <sup>A</sup>	6033 ± 1858 <sup>A</sup>	7052 ± 2472 <sup>A</sup>	6300 ± 2445 <sup>A</sup>
PhAc <sup>1</sup>	U/L	15404 ± 3351	16919 ± 3165 <sup>A</sup>	15685 ± 3542 <sup>AB</sup>	14984 ± 3386 <sup>B</sup>	14656 ± 3041 <sup>B</sup>
Genotype <sup>2</sup>						
<i>QQ</i>		68	5 <sup>A</sup>	9 <sup>A</sup>	29 <sup>B</sup>	25 <sup>B</sup>
<i>QR</i>		87	17 <sup>A</sup>	18 <sup>A</sup>	25 <sup>A</sup>	27 <sup>A</sup>
<i>RR</i>		40	16 <sup>A</sup>	13 <sup>A</sup>	5 <sup>B</sup>	6 <sup>B</sup>

<sup>1</sup>Activities of POaseNa, DZOaseNa, POase, DZOase, and hydrolysis of PhAc are expressed as micromoles substrate hydrolyzed per minute per liter of serum (U/L), means ± SD.

<sup>2</sup>Number of individuals in each race-gender group with the indicated genotype.

<sup>A</sup>Values within a row with the same superscript letter are not significantly different (p < 0.05) as determined by analysis of variance with Tukey adjustment or by analysis of chi-square ( $\chi^2$ ) with Bonferroni adjustment.

<sup>B</sup>Values within a row with the same superscript letter are not significantly different (p < 0.05) as determined by analysis of variance with Tukey adjustment or by analysis of chi-square ( $\chi^2$ ) with Bonferroni adjustment.

**Table 3**

Odds ratios of atherosclerosis associated with clinical and serum factors.

<b>Factor<sup>1</sup></b>	<b>Comparison</b>	<b>Units</b>	<b>n</b>	<b>Odds Ratio</b>	<b>95% CI</b>	<b>P-Value</b>
Age		5 years	200	1.22	(1.04,1.43)	0.0138
Gender	Male vs Female		200	4.13	(2.29,7.46)	<0.0001
Smoker	Ever vs Never		200	2.45	(1.38,4.31)	0.0023
LDLGroup	Elevated vs Normal		197	10.08	(3.75,27.08)	<0.0001
HDLGroup	Low vs Normal		196	2.66	(1.47,4.82)	0.0012
Triglyceride	Abnormal vs Normal		97	6.57	(2.70,15.97)	0.0267
BMI		5 kg/m <sup>2</sup>	198	0.75	(0.59,0.95)	0.018
POaseNa <sup>2</sup>		150 U/L	199	0.78	(0.62,0.98)	0.0326
DZOaseNa <sup>2</sup>		1000 U/L	199	0.89	(0.80,0.99)	0.0346
POase <sup>2</sup>		15 U/L	199	0.92	(0.85,0.99)	0.0334
DZOase <sup>2</sup>		1000 U/L	199	0.85	(0.75,0.97)	0.0169
PhAc <sup>2</sup>		1000 U/L	199	0.89	(0.82,0.98)	0.0128

<sup>1</sup>Factors that were included in study but not found significant at p 0.05 included race, hypertension, diabetes, family history, waist circumference, total cholesterol group, LDL/HDL ratio, genotype, DZOaseNa/POaseNa ratios, and DZOase/POase ratios

<sup>2</sup>Activities of POaseNa, DZOaseNa, POase, DZOase, and hydrolysis of PhAc are expressed as micromoles substrate hydrolyzed per minute per liter of serum (U/L), means  $\pm$  SD.

**Table 4**

Multivariable logistic regression model of clinical and serum factors associated with atherosclerosis occurrence

Factor	Comparison	Units	Odds Ratio	95% CI	P-Value
Age		5 years	1.04	(1.01,1.08)	0.0251
Gender	Male vs. Female		3.13	(1.58,6.23)	0.0011
Smoker <sup>1</sup>	Ever vs. Never		1.98	(0.98,3.99)	0.0560
Hypertension <sup>1</sup>	Yes vs. No		2.05	(0.90,4.70)	0.0888
LDLGroup	High vs. Normal		9.56	(3.17, 28.79)	<0.0001
PhAc		1000 U/L	0.89	(0.80,0.99)	0.0317

<sup>1</sup>Although the p-values for the association of smoking status and hypertension were greater than 0.05, the variables were retained in the model because each is an established risk factor for atherosclerosis

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