Evaluation of Polymorphisms in Paraoxonase 2 (PON2) Gene and Their Association with Cardiovascular-Renal Disease Risk in Mexican Americans

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Key Words

PON2 • Genetic polymorphisms • Association analyses • Systolic blood pressure • Mexican Americans

Abstract

Background/Aims: Genetic polymorphisms in the paraoxonase 2 (PON2) gene are thought to alter its activity and contribute to the development of cardiovascular and renal disease risk. The purpose of this study is to determine whether the Arg148Gly, Cys311Ser and rs12794795 polymorphisms of PON2 examined previously by others, are associated with type 2 diabetes (T2DM), and subclinical measures of cardiovascular and renal disease risk in Mexican Americans. **Methods:** Study participants (n = 848; 21 families) were genotyped for the three polymorphisms by TaqMan assay. Association between the genotypic and phenotypic data was performed by measured genotype approach as implemented in the variance component analytical tools. **Results:** The Arg148Gly variant was found to be monomorphic in our dataset. Of the phenotypes examined for association, the A/C variant located in intron-1 (rs12794795) exhibited statistically significant association only with diastolic blood pressure

(p = 0.018) after accounting for the trait-specific covariate effects. The Cys311Ser variant failed to show statistically significant association with any of the phenotypes examined. **Conclusion:** In conclusion, the variants examined at the PON2 locus in Mexican Americans do not appear to be a major contributor to T2DM, cardiovascular or renal disease risk, although they exhibited a small effect on the blood pressure values.

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Introduction

Paraoxonase 2 (PON2) is a member of the paraoxonase (PON) family, which was originally identified as an organophosphate hydrolase. The name 'PON' was derived from one of its most commonly used substrates, paraoxon, a metabolite of the insecticide parathion [1]. In addition to its role in hydrolyzing organophosphorus compounds, PON plays an important role in lipid metabolism. Genetic and biochemical studies have demonstrated that PON prevents the oxidation of low-density lipoprotein (LDL) to oxidized LDL, and preserves the function

of high-density lipoproteins by inhibiting their oxidation [2–4]. Experimental studies suggest that PON lowers the risk of coronary heart disease.

PON2 gene encoding paraoxonase 2 is located on human chromosome 7q22 [5]. PON2 is widely expressed in a variety of tissues including endothelial and smooth muscle cells as well as macrophages [6]. The antioxidative and antiatherogenic activities of PON2 have been well documented [6, 7]. Over the past decade, studies have attempted to determine the relevance of DNA sequence variants in the *PON2* locus to cardiovascular and renal disease risk. Of the several sequence variants identified in the *PON2* locus, the Arg148Gly and Cys311Ser variants have received much attention. Several epidemiological studies have associated these variants with microvascular complications of both type 1 and type 2 diabetes [8–13], coronary heart disease [14–16] and myocardial infarction [17]. In addition, an A to C variant located in intron 1 (rs12704795) was recently demonstrated to be associated with microalbuminuria [13] and albumin-to-creatinine ratio (ACR) [18].

In the present study, we investigated if these three *PON2* polymorphisms (Arg148Gly, Cys311Ser, and an intron-1 variant) previously examined by others have any relationship with type 2 diabetes (T2DM), cardiovascular and renal disease risk in a Mexican American cohort.

Patients and Methods

The San Antonio Family Heart Study (SAFHS) family member recruitment and data collection procedures were described previously [19]. Briefly, probands for the SAFHS were selected randomly from a census tract in San Antonio of low-income Mexican Americans regardless of preexisting medical conditions. A variety of metabolic, hemodynamic, anthropometric, and demographic variables were collected from more than 40 extended Mexican American families [19]. Estimation of glomerular filtration rate (eGFR) by the Modification of Diet in Renal Disease (MDRD) equation, and ACR has already been described [20]. The quantitative trait values were inverse-normalized and used in the association analyses since their raw data were non-normally distributed. The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved all procedures, and all subjects gave informed consent.

Genotyping of rs11545942 (G/A; Arg148Gly), rs7493 (C/G; Cys311Ser) and rs12704795 (A/C) polymorphisms were performed by TaqMan assay (Applied Biosystems, Foster City, Calif., USA), which was carried out on a GeneAmp PCR system 9700 (Applied Biosystems), and fluorescent signals were detected on an ABI Prism 7700 sequence detector (Applied Biosystems).

The genotypic data were checked for mendelian pedigree inconsistencies using the program INFER and GENTEST as imple-

Table 1. Clinical characteristics of the genotyped SAFHS participants¹

Variables	Mean $\pm$ SD or %
Females	63
Type 2 diabetes	22
Age, years	47.9 $\pm$ 14.8
Systolic blood pressure, mm Hg	124.4 $\pm$ 19.0
Diastolic blood pressure, mm Hg	69.8 $\pm$ 10.3
Body mass index, kg/m ²	31.8 $\pm$ 7.2
Total cholesterol, mg/dl	180.6 $\pm$ 38.5
High-density lipoprotein cholesterol, mg/dl	48.2 $\pm$ 13.6
Triglycerides, mg/dl	129.2 $\pm$ 86.6
Albumin-to-creatinine ratio	0.06 $\pm$ 0.3

¹ Sample size varies from 780 (albumin-to-creatinine ratio) to 848 (age).

mented in PEDSYS [21]. Allele frequencies were estimated using maximum likelihood techniques, which account for the pedigree structure. Linkage disequilibrium between SNPs was estimated using the r^2 values. Association analysis in our family data was carried out using the measured genotype approach (MGA) within the variance components (VC) analytical framework [22]. The VC-based approach accounts for the non-independence among family members. In this approach, VCs are modeled as random effects (e.g., additive genetic effects and random environmental effects), whereas the effects of measured covariates such as age and sex are modeled as fixed effects on the trait mean. The marker genotypes were incorporated in the mean effects model as a measured covariate, assuming additivity of allelic effects [22]. The effect of this measured genotype (i.e., association parameter) together with other covariate effects (e.g., age and sex) and VCs were estimated by maximum likelihood techniques. The hypothesis of no association is tested by comparing the likelihood of a model in which the effect of the measured genotype is estimated with a model where the effect of the measured genotype was fixed at zero. Twice the difference in the log likelihoods of these models yields a test statistic that is asymptotically distributed, approximating a χ^2 distribution with 1 degree of freedom. A p value ≤ 0.05 is considered significant. Based on the number of participants, there is 83% power to detect an association that may account for as little as 1% of the phenotypic variation. Prior to performing MGA, the quantitative transmission disequilibrium test (QTDT) was used to examine hidden population stratification [23]. All statistical techniques described above were implemented in the program SOLAR [22].

Results

Table 1 shows the clinical characteristics of the SAFHS subjects genotyped for this study. Of the genotyped individuals ($n = 848$; 21 families), the mean age of the study

Table 2. Allele and genotype frequencies of the examined variants in the *PON2* gene

Variants	Major/minor allele, %	Genotype, %			HWE	p value
rs7493	C (79)/G (21)	CC (63)	CG (31)	GG (6)	0.90	0.439
rs12704795	A (77)/C (23)	AA (60)	AC (34)	CC (6)	0.70	0.218

Table 3. Association analysis between *PON2* gene variants and cardiovascular and renal-related risk factors in SAFHS data

Phenotypes	PON2- rs12704795 p value	PON2- rs7493 p value
Type 2 diabetes ¹	0.825	0.339
Body mass index ²	0.117	0.851
Waist circumference ²	0.131	0.902
Total cholesterol ³	0.730	0.268
High density lipoprotein cholesterol ³	0.854	0.716
Triglycerides ³	0.971	0.665
Systolic blood pressure ⁴	0.963	0.767
Diastolic blood pressure ⁴	0.018	0.478
Albumin to creatinine ratio ⁵	0.582	0.942
Estimated glomerular filtration rate ⁵	0.368	0.175

¹ Data adjusted for age and sex terms.

² Data adjusted for age, sex and diabetes.

³ Data adjusted for age, sex, diabetes, duration of diabetes, BMI and lipid medication.

⁴ Data adjusted for age, sex, diabetes, duration of diabetes and antihypertensive treatment.

⁵ Data adjusted for age, sex, diabetes, duration of diabetes, systolic blood pressure and antihypertensive treatment.

subjects was 48 years, and 63% were females. The data available for each phenotype varied from 780 subjects for ACR to 848 subjects for age. Of the examined individuals from 21 families, 52, 22, and 14% had hypertension, T2DM, and albuminuria, respectively (table 1).

Of the three *PON2* polymorphisms genotyped, the rs11545942 (Arg148Gly) variant failed to be polymorphic in SAFHS data. The allele and genotype frequencies of rs7493 (C/G; Cys311Ser) and rs12704795 (A/C) are presented in table 2. As can be seen from table 2, the frequencies of the C and G alleles of rs7493 polymorphism were 79 and 21%, respectively. The frequencies of A and C alleles of rs12704795 were 77 and 23%, respectively. Genotypic data of rs7493 and rs12704795 were consistent with the Hardy-Weinberg equilibrium expectations. To guard against potential effects of hidden population stratification, we also employed the QTDT approach [23]. This approach permits a formal test of likely population

heterogeneity on a marker-specific basis [24]. Using this test, we found no evidence for such stratification. Additionally, the results of the QTDT analyses were consistent with the more powerful measured genotype analyses.

Association analysis in our family data was performed using the MGA. Of the phenotypes examined [T2DM, body mass index, systolic blood pressure, diastolic blood pressure (DBP), total cholesterol, high-density lipoprotein cholesterol, triglycerides, eGFR, and ACR] for their association with genotypes, the A/C variant (rs12704795) located in intron-1 exhibited a statistically significant association only with DBP ($p = 0.018$) after accounting for covariate effects of age, sex, diabetes, duration of diabetes, and antihypertensive medications (table 3). The non-synonymous variant (Cys311Ser) failed to show a statistically significant association with any of the phenotypes examined in this study (table 3).

Discussion

In the present study, we examined the role of selected variants in the *PON2* gene to individual susceptibility to T2DM, cardiovascular and renal disease risk in a cohort of Mexican Americans. This particular gene was selected based on the evidence that non-synonymous variants (Arg148Gly and Cys311Ser) and an intronic variant (rs12704795) within *PON2* are significantly associated with microvascular complications of both type 1 and type 2 diabetes [8–13]. The effect of the coding sequence variants of *PON2* on its activity or concentration is unknown. However, coding sequence variants may impact *PON2* activity and influence lipid metabolism pathways (e.g., lipoprotein lipase or hepatic lipase) thereby contributing to changes in the levels and composition of lipids and lipoproteins. Furthermore, genetic association studies examining the above-mentioned polymorphisms with microvascular complications of diabetes have been mostly conducted in Caucasian and Asian populations. Epidemiological studies examining the role of these polymorphisms in Mexican Americans are very limited except for one recent study where we investigated these *PON2* polymorphisms in relation to cardiovascular and renal disease risk in another Mexican American cohort enriched with T2DM [18].

In the present study, the minor allele frequencies observed both the rs7493 (Cys311Ser) and rs12704795 polymorphisms were similar to those we reported [18] previously in another Mexican American cohort – the San Antonio Family Diabetes/Gallbladder Study (SAFDGS) – which also examined the role of these three *PON2* variants and their relevance to cardiovascular and renal disease risk. However, the association analysis from this study does not support the findings of our previous study and others, which demonstrated the presence of significant association between Cys311Ser polymorphisms and variation in ACR [13–18]. This may be either due to the difference in the sample size or the family ascertainment criteria for each study. While the probands for SAFHS were ascertained based on low-income Mexican Americans regardless of preexisting medical conditions [19], the probands for SAFDGS were ascertained on T2DM [25].

In view of the importance of *PON2* in lipid metabolism, the relationship between Cys311Ser polymorphisms of *PON2* and plasma lipoproteins has been examined by other studies [8, 15]. These investigators observed that the carriers of Ser311 and Cys311Ser had significantly higher plasma total and LDL cholesterol than subjects carrying

the Cys311 genotype. However, our association analyses failed to find any statistically significant association between the Cys311Ser polymorphisms and serum lipid and lipoprotein levels (table 3). Similar to our findings, other studies, including our previous report [18], could not demonstrate significant association of Cys311Ser polymorphisms with lipoprotein levels [2]. None of these studies examined the levels of the oxidized lipids.

Association analysis indicated the presence of a significant association between the A/C variant located in intron-1 and DBP. Although the mechanism responsible for this association needs to be elucidated, the genetic effect however appears to be modest. The current study has advantages in that it measures multiple cardiovascular-renal-related traits in the same individuals in a relatively large sample of Mexican American families. The present study has limitations in that it attempts to replicate the three variants examined previously by several studies of the *PON2* gene and has not attempted comprehensive tagging of all common variation within the *PON2*. In addition, the eGFR estimated by the MDRD formula has not been validated in the Mexican Americans. However, the MDRD equation is commonly used to estimate GFR and has been employed in several genetic studies [26–28]. It is to be noted that while allowing for multiple testing of SNPs preserves the significance of the one association we found, it does not consider corrections for tests across multiple phenotypes since each phenotype can be considered to reflect a unique hypothesis.

In conclusion, the present study adds evidence to other reports that the variants examined at *PON2* locus do not appear to be a major contributor to T2DM, cardiovascular and renal disease risk, although it exhibited a small effect on DBP values in this cohort.

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References

- Costa LG, Richter RJ, Li WF, Cole T, Guizzetti M, Furlong CE: Paraoxonase (PON1) as a biomarker of susceptibility for organophosphate toxicity. *Biomarkers* 2003;8:1–12.
- Mackness MI, Arrol S, Abbott CA, Durrington PN: Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein-associated paraoxonase. *Atherosclerosis* 1993;104:129–135.
- Watson AD, Berliner JA, Hama SY, La Du BN, Faut K, Fogelman AM: Protective effect of high-density lipoprotein associated paraoxonase – inhibition of the biological activity of minimally oxidised low-density lipoprotein. *J Clin Invest* 1995;96:2882–2891.
- Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parma SL, La Du BN: Paraoxonase inhibits high-density lipoprotein oxidation and preserves its functions. A possible peroxidative role for paraoxonase. *J Clin Invest* 1998;101:1581–1590.
- Primo-Parma SL, Sorenson RC, Teiber J, La Du BN: The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics* 1996;33:498–507.
- Ng CJ, Wadleigh DJ, Gangopadhyay A, Hama S, Grijalva VR, Navab M, Fogelman AM, Reddy ST: Paraoxonase-2 is a ubiquitously expressed protein with antioxidant properties and is capable of preventing cell-mediated oxidative modification of low-density lipoprotein. *J Biol Chem* 2001;276:44444–44449.
- Rosenblat M, Draganov D, Watson CE, Bisgaier CL, La Du BN, Aviram M: Mouse macrophage paraoxonase 2 activity is increased whereas cellular paraoxonase-3 activity is decreased under oxidative stress. *Arterioscler Thromb Vasc Biol* 2003;23:468–474.
- Hegele RA: Paraoxonase genes and disease. *Ann Med* 1999;31:217–224.
- Pinizzotto M, Castillo E, Fiaux M, Tember E, Gaillard RC, Ruiz J: Paraoxonase-2 polymorphisms are associated with nephropathy in type 2 diabetes. *Diabetologia* 2001;44:104–107.
- Hodgkinson AD, Millward BA, Demaine AG: Commentary to letter. *Diabetologia* 2002;45:933–935.
- Canani LH, Araki S, Warram JH, Krolewski AS: Commentary to letter. *Diabetologia* 2001;44:1062–1069.
- Kao Y, Donaghue KC, Chan A, Bennetts BH, Knight J, Silink M: Paraoxonase gene cluster is a genetic marker for early microvascular complications in type 1 diabetes. *Diabet Med* 2002;19:212–215.
- Sanghera DK, Aston CE, Saha N, Kamboh MI: DNA polymorphisms in two paraoxonase genes (PON1 and PON2) are associated with the risk of coronary heart disease. *Am J Hum Genet* 1998;62:36–44.
- Leus FR, Zwart M, Kastelein JJ, Voorbij HA: PON2 gene variants are associated with clinical manifestations of cardiovascular disease in familial hypercholesterolemia patients. *Atherosclerosis* 2001;154:641–649.
- Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, Sopko G, Pauly DF, Merz CN, Kamboh MI, WISE Study Group: Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) Study. *Am J Hum Genet* 2003;72:13–22.
- Martinelli N, Girelli D, Olivieri O, Stranieri C, Trabetti E, Pizzolo F, Friso S, Tenuti I, Cheng S, Grow MA, Pignatti PF, Corrocher R: Interaction between smoking and PON2 Ser311Cys polymorphism as a determinant of the risk of myocardial infarction. *Eur J Clin Invest* 2004;34:14–20.
- Calle R, McCarthy MI, Banerjee P, Zeggini E, Cull CA, Thorne KI, Wiltshire S, Terra S, Meyer D, Richmond J, Mancuso J, Milos P, Fryburg D, Holman RR: Paraoxonase 2 (PON2) polymorphisms and development of renal dysfunction in type 2 diabetes: UKPDS 76. *Diabetologia* 2006;49:2892–2899.
- Thameem F, Puppala S, Arar NH, Farook VS, Stern MP, Blangero J, Duggirala R, Abboud HE: The Cys(311)Ser polymorphism of paraoxonase 2 is associated with albumin-to-creatinine ratio in Mexican Americans. *Nephrol Dial Transplant* 2008;23:416–417.
- MacCluer JW, Stern MP, Almasy L, Atwood LA, Blangero J, Comuzzie AG, Dyke B, Haffner SM, Henkel RD, Hixson JE, Kammerer CM, Mahaney MC, Mitchell BD, Rainwater DL, Samollow PB, Sharp RM, VandeBerg JL, Williams JT: Genetics of atherosclerosis risk factors in Mexican Americans. *Nutr Rev* 1999;57:S59–S65.
- Arar NH, Voruganti VS, Nath SD, Thameem F, Bauer R, Cole SA, Blangero J, Maccluer JW, Comuzzie AG, Abboud HE: A genome-wide search for linkage to chronic kidney disease in a community-based sample: the SAFHS. *Nephrol Dial Transplant* 2008;10:3184–3191.
- Dyke B: PEDSYS, a Pedigree Data Management System. User's Manual. Population Genetics Laboratory Technical Report No. 2, ed 2. San Antonio, Southwest Foundation for Biomedical Research, 1994, pp 1–226.
- Almasy L, Blangero J: Exploring positional candidate genes: linkage conditional on measured genotype. *Behav Genet* 2004;34:173–177.
- Abecasis GR, Cookson WO, Cardon LR: Pedigree tests of transmission disequilibrium. *Eur J Hum Genet* 2000;8:545–551.
- Havill LM, Dyer TD, Richardson DK, Mahaney MC, Blangero J: The quantitative trait linkage disequilibrium test: a more powerful alternative to the quantitative transmission disequilibrium test for use in the absence of population stratification. *BMC Genet* 2005;6:S91.
- Puppala S, Dodd GD, Fowler S, Arya R, Schneider J, Farook VS, Granato R, Dyer TD, Almasy L, Jenkinson CP, Diehl AK, Stern MP, Blangero J, Duggirala R: A genome-wide search finds major susceptibility loci for gallbladder disease on chromosome 1 in Mexican Americans. *Am J Hum Genet* 2006;78:377–392.
- Puppala S, Arya R, Thameem F, Arar NH, Bhandari K, Lehman DM, Schneider J, Fowler S, Farook VS, Diego VP, Almasy L, Blangero J, Stern MP, Duggirala R, Abboud HE: Genotype by diabetes interaction effects on the detection of linkage of glomerular filtration rate to a region on chromosome 2q in Mexican Americans. *Diabetes* 2007;56:2818–2828.
- Schelling JR, Abboud HE, Nicholas SB, Pahl MV, Sedor JR, Adler SG, Arar NH, Bowden DW, Elston RC, Freedman BI, Goddard KA, Guo X, Hanson RL, Ipp E, Iyengar SK, Jun G, Kao WH, Kasinath BS, Kimmel PL, Klag MJ, Knowler WC, Nelson RG, Parekh RS, Quade SR, Rich SS, Saad MF, Scavini M, Smith MW, Taylor K, Winkler CA, Zager PG, Shah VO; Family Investigation of Nephropathy and Diabetes Research Group: Genome-wide scan for estimated glomerular filtration rate in multi-ethnic diabetic populations: the Family Investigation of Nephropathy and Diabetes (FIND). *Diabetes* 2008;57:235–243.
- Freedman BI, Bowden DW, Rich SS, Xu J, Wagenknecht LE, Ziegler J, Hicks PJ, Langefeld CD: Genome-wide linkage scans for renal function and albuminuria in type 2 diabetes mellitus: the Diabetes Heart Study. *Diabet Med* 2008;25:268–276.