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# Serum paraoxonase activity is associated with variants in the PON gene cluster and risk of Alzheimer disease

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#### Abstract

Previous studies have shown association of single nucleotide polymorphisms (SNPs) in 3 contiguous genes (PON1, PON2, and PON3) encoding paraoxonase with risk of Alzheimer disease (AD). We evaluated the association of serum paraoxonase activity measured by phenyl acetate (PA) and thiobutyl butyrolactone (TBBL) with risk of AD and with 26 SNPs spanning the PON gene cluster in 266 AD cases and 306 sibling controls from the MIRAGE study. The odds of AD (adjusted for age, gender, and ethnicity) increased 20% for each standard deviation decrease in PA or TBBL activity. There were association signals with activity in all 3 genes. Haplotypes including SNPs spanning the PON genes were generally more significant than haplotypes comprising SNPs from 1 gene. Significant interactions were observed between SNP pairs located across the PON cluster with either serum activity measure as the outcome, and between several PON SNPs and PA activity with AD status as the outcome. Our results suggest that low serum paraoxonase activity is a risk factor for AD. Furthermore, multiple variants in PON influence serum paraoxonase activity and their effects may be synergistic. © 2012 Elsevier Inc. All rights reserved.

#### 1. Introduction

Paraoxonases are a family of enzymes with ester hydrolyzing activity that are able to hydrolyze organophosphate compounds and platelet activating factor, impede lipoprotein oxidation, inhibit macrophage cholesterol biosynthesis, stimulate cholesterol efflux from macrophages, and delay atherosclerosis (Draganov et al., 2005; Harel et al., 2004; Stoltz et al., 2009). They are encoded by the 3 contiguous genes (PON1, PON2, and PON3) sharing considerable sequence identity and spanning  $\sim 150$  kb on chromosome 7q21.3. These enzymes have similar but not identical activities (Draganov et al., 2005). While all paraoxonase enzymes are capable of catalyzing the hydrolysis and condensation of ester bonds in a broad range of substrates (Teiber et al., 2003), their substrate specificity and catalytic activities differ. PON1 and PON3 are found in serum whereas PON2 is primarily an intracellular enzyme (Stoltz et al., 2009). In vitro, paraoxonase activity in serum can be measured using colorimetric assays (Browne et al., 2007; Furlong et al., 1989; Nozawa et al., 1980) utilizing a variety of indicator substrates (La Du et al., 1993; Lockridge et al., 1980; Smolen et al., 1991) including thiobutyl butyrolactone (TBBL) and phenyl acetate (PA). The PA assay, which measures an arylesterase activity of paraoxonase, is a widely used assay but may not represent a native physio-

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logical activity (Khersonsky et al., 2006). The TBBL assay measures a lactonase activity of paraoxonase, which is important in regulating expression of virulence factors and inducing a host inflammatory response (Draganov et al., 2005; Khersonsky and Tawfik, 2005). PON activity in serum is strongly influenced by some coding and regulatory sequence variations in PON1 (Adkins et al., 1993; Brophy et al., 2001a, 2001b; Browne et al., 2007; Deakin et al., 2003), however, a substantial portion of the overall phenotypic variance in PON activity between individuals remains unexplained by any single defined DNA polymorphism (Brophy et al., 2001a, 2001b; Chen et al., 2005; Deakin et al., 2003; Saeed et al., 2007).

Serum paraoxonase activity phenotypes and polymorphisms in this gene cluster are associated with a variety of atherosclerosis-driven vascular outcomes including coronary artery disease, stroke, and oxidative stress (Aydin et al., 2006; Bhattacharyya et al., 2008; Jarvik et al., 2000; Kim et al., 2009; Leviev et al., 2001; Mackness et al., 1991, 1998, 2003; McElveen et al., 1986; Yildiz et al., 2008), although their reliability as predictors of vascular disease risk has not been firmly established. Serum paraoxonase activity was previously examined in relation to Alzheimer's disease (AD) in 2 studies: (1) Paragh et al. (2002) observed that serum paraoxonase activity is reduced in both AD and vascular dementia subjects relative to controls; and (2) Dantoine et al. (2002a, 2002b) found that serum paraoxonase activity is reduced in vascular dementia but not significantly altered in AD relative to controls; however the number of controls was fairly small. Thus the relation between PON polymorphisms, PON serum activity, and susceptibility to AD is unclear and is important for the evaluation of PON as a risk factor for AD. Previously, we identified association of AD with several single nucleotide polymorphisms (SNPs) in the PON gene cluster, in particular an SNP (rs705381) in the PON1 promoter (Erlich et al., 2006). In this study, we evaluated the association of paraoxonase activity measured by the TBBL and PA assays in serum with a comprehensive set of PON SNPs and the interaction of the serum paraoxonase measures and PON SNPs on risk of AD.

# 2. Methods

#### 2.1. Subject recruitment and evaluation

This study included 266 AD cases and 306 sibling controls from the MIRAGE study, a family-based genetic epidemiological study of AD described in detail elsewhere (Green et al., 2002). Briefly, AD patients and their siblings were recruited from 14 clinical centers in the United States, Canada, Germany, and Greece. Sibships were ascertained through a single affected proband with probable AD according to National Institute of Neurological and Communicative Disorders and Stroke - Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984). Cognitive status of individuals

Table 1	
Sample	characteristics

Population	AD status	п	Mean age	% male
African Americans	AD cases	36	75.1	25.0
	Unaffected sibs	46	69.5	34.8
Caucasians	AD cases	204	73.8	45.1
	Unaffected sibs	230	69.5	40.5
Asian Americans	AD cases	26	77.3	61.5
	Unaffected sibs	30	84.3	40.0
Total	Total affected	266		
	Total unaffected	306		

identified as nondemented was confirmed by a score 86 or higher on the modified Telephone Interview of Cognitive Status (Roccaforte et al., 1992). Age at onset of AD was defined as the age of earliest reported symptoms by proxy. Subjects were classified according to self-reported ethnicity as Caucasian, African American, or Asian American. Nonfasting blood specimens were obtained for DNA and biochemical analyses. Only persons who gave written informed consent are included in this study. Study protocols were approved by Institutional Review Boards at each recruitment site. Subject characteristics are shown in Table 1.

# 2.2. SNP selection and genotyping

Twenty-nine SNPs distributed across the PON gene cluster region were selected as previously described (Erlich et al., 2006). Genotyping of genomic DNA extracted from peripheral blood lymphocytes was performed using SNP assays obtained from Applied Biosystems, Inc. (Foster City, CA), on an ABI 7900 (real-time) platform using the manufacturer's protocols. Duplicate wells were scattered on DNA template plates. The duplicate discordance rate did not exceed 5% except for 2 samples which were subsequently excluded from all analyses. The overall genotype call rate was >95% for all SNPs typed. SNPs were assessed for Hardy-Weinberg equilibrium (HWE) in unrelated, unaffected sibs within each ethnic group, and excluded from further analysis in that ethnic group if the test was significant at the 0.05 level. Three SNPs were monomorphic and excluded from further analysis. Characteristics of the 26 informative and 3 uninformative SNPs are shown in Table 2.

## 2.3. Phenyl acetate activity assay

Paraoxonase activity in individual serum samples was assayed using kinetic spectroscopy of the hydrolysis reaction of phenyl acetate as previously described (Brophy et al., 2001b), with small modifications to allow for the use of a 96-well plate configuration. Reactions in a final volume of 300  $\mu$ L, in quadruplicate, were performed in ultraviolet (UV)-clear microtiter plates (Greiner, Kremsmünster, Austria). Each well contained serum (at a final dilution of 1200-fold or higher), 9 mM Tris-Cl, pH 8.0, 0.9 mM CaCl2, and 3.2 mM phenylacetate. Reactions were initiated by the addition of 30  $\mu$ L of 10× substrate solution to 270  $\mu$ L of

Table 2 SNPs genotyped in the PON gene cluster

SNP number	NCBI reference	Position (NCBI_35)	Genomic context or	Gene	Minor allele (frequency)			
			predicted function		African Americans	Caucasians	Asian Americans	
1	rs2237582	94578851	Intron	PON1	A (0.29)	G (0.30)	A (0.30)	
2	rs2269829	94580780	Intron	PON1	G (0.49)	G (0.29)	A (0.29)	
3	rs662	94582097	R192Q	PON1	T (0.32)	C (0.29)	T (0.28)	
4	rs2299255	94583437	Intron	PON1	C (0.10)	C (0.15)	C (0.10)	
5	rs13306698	94585433	R160G	PON1	A $(0.00)^{a}$	A $(0.00)^{a}$	A $(0.00)^{a}$	
6	rs1157745	94585689	Intron	PON1	G (0.32)	T (0.30)	G (0.28)	
7	rs854560	94590735	L55M	PON1	T (0.20)	T (0.35)	T (0.1)	
8	rs854565	94592995	Intron	PON1	A (0.35)	A (0.31)	A (0.18)	
9	rs705379	94598546	Promoter (-107) <sup>b</sup>	PON1	A (0.12)	A (0.49)	G (0.49)	
10	rs705381	94598600	Promoter (-161) <sup>b</sup>	PON1	T (0.42)	T (0.26)	T (0.08)	
11	rs2375001	94609291	Intergenic	Intergenic	T (0.22)	T (0.23)	T (0.13)	
12	rs1859121	94621618	Intergenic	Intergenic	T (0.44)	C (0.44)	T (0.14)	
13	rs2074352	94634324	Intron	PON3	T (0.06)	T (0.19)	C (0.37)	
14	rs3757708	94641564	Intron	PON3	T (0.43)	G (0.44)	T (0.14)	
15	rs2375003	94646184	N107D	PON3	A (0.00) <sup>a</sup>	A (0.00) <sup>a</sup>	A (0.00) <sup>a</sup>	
16	rs978903	94648818	Intron	PON3	A (0.43)	G (0.43)	A (0.14)	
17	rs10487132	94664956	Intron	PON3	G (0.14)	G (0.45)	G (0)	
18	rs2072200	94670811	Promoter	PON3	C (0.07)	C (0.18)	G (0.4)	
19	rs6954345	94679426	C311S	PON2	C (0.29)	C (0.24)	C (0.24)	
20	rs3735586	94680233	Intron	PON2	T (0.31)	T (0.24)	T (0.24)	
21	rs10487133	94680601	Intron	PON2	G (0.20)	G (0.10)	G (0.08)	
22	rs2375005	94681527	Intron	PON2	T (0.46)	A (0.44)	T (0.09)	
23	rs987539	94681643	Intron	PON2	C (0.36)	T (0.45)	C (0.09)	
24	rs6961624	94682312	Intron	PON2	G (0.30)	G (0.24)	G (0.24)	
25	rs2299263	94685062	Intron	PON2	T (0.29)	T (0.25)	T (0.24)	
26	rs1034809	94696303	Intron	PON2	A (0.24)	A (0.25)	A (0.35)	
27	rs13306699	94698531	F31S	PON2	A (0.00) <sup>a</sup>	A (0.00) <sup>a</sup>	A (0.00) <sup>a</sup>	
28	rs2286233	94698908	Intron	PON2	T (0.24)	T (0.10)	T (0.09)	
29	rs2299267	94706572	Intron	PON2	G (0.13)	G (0.14)	G (0.41)	

Key: SNP, single nucleotide polymorphism; NCBI, National Center for Biotechnology Information.

<sup>a</sup> Minor allele not observed in study sample.

<sup>b</sup> Relative to PON1 translation initiation point in build 35.1.

diluted serum using an automatic 96-channel multidispenser (Labcyte, Sunnyvale, CA). Plates were read at 270 nm (with 49-second intervals between reads) in a plate reader ( $\mu$  Quant; Bio-Tek, Inc., Winsooki, VT). A linear range for the assay was first established using a dilution series of a mixture of 10 de-identified serum samples, unrelated to the study, obtained from the clinical laboratory. Study samples were first tested at 1200-fold final dilution and retested at higher fold if they were outside of this linear range.

## 2.4. Thiobutyl butyrolactone (TBBL) assay

The TBBL hydrolyzing activity of paraoxonase was assayed in individual serum samples using kinetic spectroscopy of the hydrolysis reaction of thiobutyl butyrlactone as described elsewhere (Khersonsky and Tawfik, 2006), with minor modifications. Reactions in a final volume of 200  $\mu$ L, in triplicate, were performed in ultraviolet (UV)-clear microtiter plates (Greiner). Each well contained serum (at a final dilution of 1200-fold), 22 mM Tris-Cl, pH 8.0, 1.0 mM CaCl<sub>2</sub>, 0.5 mM 5,5'-dithiobis 2-nitrobenzoic acid (DTNB = Ellman's reagent), 0.5% dimethylsulfoxide (DMSO), 1% acetonitrile, and 0.2 mM TBBL. Stock solutions were 200 mM TBBL in acetonitrile and 100 mM DTNB in dimethylsulfoxide (DMSO). Reactions were initiated by the addition of (in this order) DTNB and (5 minutes later) TBBL, to diluted serum using an automatic 96-channel multidispenser (Labcyte). Plates were read at 412 nm (at 49-second intervals between reads) in a plate reader ( $\mu$  Quant; Bio-Tek, Inc.).

#### 2.5. Determination of PON serum activity phenotypes

Both assays proceeded at linear rates during the first 10 minutes from the start of the reactions, allowing rates to be obtained by linear regression based on the first 12 data points. Thus, the slope of the curve during the linear phase of the reaction (first 12 data points) constituted the measurement. Immediately after completion of the kinetic measurement, each plate was read once at 977 nm and once at 900 nm for path length correction. The path length was determined as follows; path =  $(A_{977} - A_{900})^{\text{well}}/0.18$ . The slope for each sample was estimated from the time course plot using linear regression of the path-length corrected absorbance over the initial 12 data points of the reaction. The rate of spontaneous hydrolysis (without serum) was subtracted to obtain the net rate. For PA, net rates were converted to standard units of aryl esterase activity (molar activity per minute per liter of serum) using the published extinction coefficient of phenol in aqueous solution at 270



Fig. 1. PON serum activity phenotypes were measured by reacting individual serum samples with either thiobutyl butyrolactone (TBBL) or phenyl acetate (PA) and monitoring the rate of product accumulation (hydroxy butyric acid for TBBL; phenol for PA). Selected representative time-course plots for each of the 2 activity phenotypes are shown including the minimum, maximum, and quartile-boundary observations; spontaneous hydrolysis (no serum) which was low in both assays is also shown.

nm (1.31 optical density [OD]  $\text{mM}^{-1}$  cm<sup>-1</sup>). For TBBL, the results are reported in units of OD per minute without further conversions. Control reactions containing substrate but no serum were included on each plate as negative control to ensure that the rate of spontaneous reaction is sufficiently low. Time course plots for samples laying on quartile boundaries and a negative control are shown in Fig. 1.

#### 2.6. Statistical methods

The distributions of PON serum activity phenotypes (PA and TBBL) were examined using SAS (version 9, SAS Institute, Cary, NC) and are shown for AD cases and controls for each ethnic group in Table 1. Association of these PON serum activity phenotypes with individual SNPs and haplotypes was evaluated using family-based association tests in PBAT version 1.32 (www.biostat.harvard.edu/ ~clange/pbat3/default.htm) under the null hypothesis of no linkage and no association. These analyses were adjusted for age at blood draw, gender, and AD status and assumed an additive genetic model with the minor allele as the referent. All possible 3-SNP combinations (2600 combinations) were evaluated by haplotype analysis. Because this group of SNPs spans 2 haplotype blocks (Erlich et al., 2006; see Supplementary Fig. 1), many of these statistical haplotypes do not necessarily correspond to ancestral haplotypes. Haplotype-specific tests were performed for SNP combinations that returned a haplotype global test *p*-value smaller than the Bonferroni threshold for multiple testing (i.e.,  $\leq 0.05/2600 = 0.000019$ ). Asymptotic *p*-values are reported for all tests. The effect of serum paraoxonase activity (PA and TBBL) on the odds of AD was estimated using generalized estimating equations (GEE) to account for sibship correlations, adjusting for gender and age (age at first symptoms for AD patients; age at interview for nondemented siblings). Analyses were performed in each ethnic group separately as well as in all ethnic groups combined (in which case ethnicity was adjusted for in the model). Regression coefficients were multiplied by the standard deviation of TBBL or PA and exponentiated to obtain the odds ratio per standard deviation (SD).

# 3. Results

#### 3.1. Association of TBBL with PON SNPs

In the single-SNP analyses, 15 SNPs scattered throughout the PON cluster were significantly associated with TBBL after Bonferroni correction (Fig. 2A, Supplementary Table 1). The most significantly associated SNP ( $p = 1.1 \times$  $10^{-6}$ ) was SNP 3 (rs662), which is a PON1 coding polymorphism (Q192R) with a known effect on the catalytic properties of paraoxonase (Browne et al., 2007). In haplotype analyses, 6.3% of all 3 SNP combinations were significant after Bonferroni correction (i.e., threshold of global  $p \leq 0.000019$ ). The most significantly associated haplotypes from among all 2600 combinations tested are displayed in Fig. 2B. These haplotypes frequently contained SNPs 1, 3, 6, or 9 (located in PON1) as well as SNPs 21 or 28 (located in PON2). SNPs 1, 3, 6, and 9 were significant individually (Fig. 2A) and showed an allelic pattern of association in haplotypes, which coincided with effect direction (Fig. 2B and D). Inclusion of either PON2 SNP 21 or 28 (which were not significant individually) increased the significance of the haplotype results; however the same alleles were present on both risk and protective haplotypes. Examination of 3 SNP haplotypes restricted to the PON3-PON2 region (SNPs 11-29) revealed a significantly associated haplotype in PON2 (Fig. 2C). This haplotype included SNPs 20 and 26, which were consistently present in the most significant haplotypes in this region and exhibited an



Fig. 2. Association of PON single nucleotide polymorphisms (SNPs) and haplotypes with thiobutyl butyrolactone (TBBL) activity. (A) Single SNP tests. Columns represent the negative log10 of the *p*-value obtained for each SNP. Red horizontal lines indicate significance thresholds. The PON gene cluster is drawn to scale showing the locations of the PON genes and the SNPs genotyped. SNPs 1-9 and SNPs 11-29 are located in separate haplotype blocks (see Supplementary Fig. 1). (B) Most significant haplotypes among all possible SNP combinations. (C) Most significant haplotypes among SNPs 1-29. (D) Most significant haplotypes among SNPs 1-9. (B–D) Haplotypes with the most significant global and specific *p*-values in the designated group of SNPs are shown. # fams, the number of informative families included in the test; freq, haplotype frequency; globP, global test *p*-value; specP, haplotype specific *p*-value.

allelic pattern of association that discriminated high- from low-expressing haplotypes.

## 3.2. Association of PA with PON SNPs

Three SNPs were significantly associated with serum paraoxonase activity as measured by the phenyl acetate assay (Fig. 3, Supplementary Table 2). The most significantly associated SNP (p = 0.000007) was SNP 9 (rs705379) which is a PON1 promoter polymorphism (-107), with a functionally proven effect on transcription (Brophy et al., 2001a, 2001b; Deakin et al., 2003). Overall, the results of haplotype analysis for activity on PA were similar to those observed for activity on TBBL. In haplotype analysis of all 3 SNP combinations, 83 combinations (3.2%) were significant after Bonferroni correction. The most significantly associated haplotypes for PA (Fig. 3B) are a subset of those observed for TBBL (Fig. 2B). Examination of region-specific SNP combinations (SNPs 1–9; SNPs 11–29) failed to identify significantly associated



Fig. 3. Association of PON single nucleotide polymorphisms (SNPs) and haplotypes with phenyl acetate (PA) activity. (A) Single SNP tests. (B) Most significant haplotypes among all possible SNP combinations. (C) Most significant haplotypes among combinations of SNPs 1, 2, 3, 6, 9, 20, and 26. See Fig. 2 for additional details.

haplotypes; however, haplotypes containing SNP 9 and SNP 26 from different haplotype blocks (Supplementary Fig. 1) were among the most significantly associated with PA (Fig. 3C) and were distinct from the top results for TBBL.

# 3.3. Interaction of PON SNPs with serum paraoxonase activity measures

The results of haplotype analyses suggest that both PON1 and PON2 may harbor genetic determinants affecting serum paraoxonase activity. Likelihood ratio (LR) tests were performed to test the hypothesis that a model containing a term for interaction of 2 SNPs better explains the effect of PON genes than a model without interaction. A total of 325 SNP pair models (1 for each unique pair of 26 SNPs) using GEE adjusting for age, gender, AD status, and ethnicity were tested thus requiring an LR test statistic to be significant at p < 0.00015. For TBBL activity, SNP  $\times$  SNP interaction terms in the models containing rs2299255 and rs2072200 or rs705379 and rs10487132 (located in different haplotype blocks spanning PON1 and PON3), and the models containing rs1034809 and either rs2074352 or rs2072200 (from PON 3 and PON 2 in the same haplotype block), were significant after Bonferroni correction, suggesting that the dependence of serum activity on sequence variation in the PON gene cluster may involve  $\text{SNP} \times \text{SNP}$  interaction. No significant interactions were observed for PA activity after Bonferroni correction (see Supplementary Table 3).

# 3.4. Comparison of allelic associations for serum paraoxonase activity and AD

The alleles associated with low activity in single SNP tests were the same for TBBL and PA for all 26 SNPs (Fig. 4). Furthermore, there was considerable concordance between the alleles associated with low activity and those associated with higher risk for AD. This concordance extended throughout regions of PON1 (SNPs 1–10) and PON2 (SNPs 20–28), including all SNPs with significant *p*-values and/or involvement in significant haplotypes.

## 3.5. Association of serum paraoxonase activity with AD

TBBL and PA measures of serum paraoxonase activity were significantly lower in AD cases compared with sibling controls. In multivariate analysis adjusted for age, gender and ethnicity, the odds of AD increased 1.23-fold (95% confidence interval [CI] = 1.07-1.41; p = 0.003) and 1.19-fold (95% CI = 1.04-1.3; p = 0.012) per standard devia-



Fig. 4. Allelic patterns of association for single nucleotide polymorphisms (SNPs) with serum paraoxonase activity phenotypes and Alzheimer's disease (AD) risk. Alleles associated with lower activity (thiobutyl butyrolactone [TBBL] or phenyl acetate [PA]) and higher risk of AD (according to test results of individual SNPs) are shown for each SNP. Dark background, regions of consensus; orange font, significantly associated SNPs ( $p < 1.9 \times 10^{-5}$  for TBBL and PA;  $p \leq 0.05$  for AD).

tion decrease in paraoxonase activity level for TBBL and PA, respectively (Table 3). The direction of this association was similar across ethnic groups analyzed separately, although the effect size varied somewhat and the estimates were not significant in every group (Supplementary Fig. 2).

# 3.6. Joint effects of PON SNPs and serum paraoxonase activity on AD risk

Prior studies have shown that the effect of paraoxonase on various vascular health outcomes is a combination of quantity, measured as the level of activity present in the serum, and quality, measured as structural DNA variation (Richter and Furlong, 1999). We therefore hypothesized that the effect of serum activity on AD risk might be dependent on SNP genotypes. To test this hypothesis, for each SNP we fitted 2 GEE models (with AD as the outcome) that included the SNP, TBBL or PA activity, age, gender, and ethnicity. We also considered models that included an interaction term for the SNP and activity (SNP imesTBBL or SNP  $\times$  PA). Among models without the interaction term, the significance of the association of either serum paraoxonase activity measure with AD risk was not appreciably diminished by inclusion of PON1 SNPs rs2237582, rs662, rs1157745, or rs705379 (Table 4). However, serum paraoxonase activity was no longer associated with AD risk after adjustment for the effects of the other PON1 SNPs or any SNPs in PON3 and PON2.

Further insight into the joint effects of variation in the PON SNPs and serum paraoxonase level on AD risk can be gleaned from models allowing for interaction of these main effects. With the exception of rs2299255, none of the interactions involving PON1 SNPs and TBBL activity were significant (Table 4). In contrast, significant interactions were found for PA activity with 9 PON3 and PON2 SNPs, including the missense variant C311S. The model including rs3735586 and PA activity was the only 1 showing significant main effects and interaction terms after Bonferroni correction. To characterize this interaction further, we fitted GEE models adding the SNP, PA activity and their interaction in a stepwise manner (Table 5). The full model including the interaction of rs3735586 and PA activity was a significant improvement over the model lacking the interaction term. Fig. 5 shows that among persons with PA activity levels below 120 units, the risk of AD is higher among rs3735586 A/T heterozygotes than T/T homozygotes. For values of PA activity above 120 units, T/T subjects have a greater risk for AD than C/T subjects.

# 4. Discussion

In this study, the relation between serum paraoxonase activity and AD was examined by measuring 2 serum PON activity phenotypes (TBBL and PA) in 266 AD patients and 306 nondemented sib controls from 3 ethnic groups. The odds of AD (adjusted for age, gender, and ethnicity) increased 20% for each standard deviation decrease in PA or TBBL activity. Analyses of individual SNPs revealed association signals for serum activity in all 3 PON genes. Hap-

Table 3								
Association	of serum	paraoxonase	activity	measures	with	Alzheimer's	disease	risk

Group	Thiobutyl bu	tyrolactone				Phenyl acetate						
	Mean (SD)					Mean (SD)						
	Cases	Controls	Odds ratio <sup>a</sup>	95% CI	<i>p</i> -value	Cases	Controls	Odds ratio <sup>a</sup>	95% CI	<i>p</i> -value		
Caucasian	22.56 (5.30)	23.48 (5.78)	1.22	1.04-1.44	0.013	126.61 (35.68)	128.02 (37.90)	1.07	0.91-1.25	0.42		
African American	27.80 (7.71)	27.58 (6.10)	1.16	0.83-1.62	0.38	146.28 (53.47)	161.23 (49.69)	1.48	1.005-2.17	0.046		
Asian American	22.59 (5.66)	25.18 (6.56)	1.78	0.99-3.21	0.053	118.65 (35.61)	138.89 (45.24)	1.14	0.63-2.05	0.68		
Total	23.28 (5.97)	24.24 (6.07)	1.23	1.07-1.41	0.003	128.49 (39.10)	133.96 (42.12)	1.19	1.04-1.36	0.012		

Key: CI, confidence interval.

<sup>a</sup> Odds of Alzheimer's disease per standard deviation unit decrease in activity level, adjusted for age, sex, and ethnicity (in unstratified analysis).

Table 4						
Interaction of paraoxonase	activity ar	nd SNP	genotype on	Alzheimer's	disease	risk

Gene	Marker	Marker SNP number	With interaction term						Without interaction term			
			PA mode <i>p</i> -values	PA model <i>p</i> -values		TBBL me <i>p</i> -values	odel		PA model <i>p</i> -values		TBBL mo <i>p</i> -values	odel
			Activity	SNP	Interaction	Activity	SNP	Interaction	Activity	SNP	Activity	SNP
PON1	rs2237582	1	1.3E-02	4.1E-02	N.S.	1.4E-02	N.S.	N.S.	4.9E-03	2.3E-02	1.7E-04	1.4E-03
	rs2269829	2	9.1E-03	N.S.	N.S.	2.9E-03	3.2E-02	N.S.	8.5E-03	N.S.	2.5E-03	N.S.
	rs662	3	N.S.	1.7E-02	N.S.	N.S.	N.S.	N.S.	3.7E-03	2.9E-02	2.7E-04	1.6E-03
	rs2299255	4	N.S.	3.8E-02	2.2E-02	N.S.	2.4E-03	2.3E-03	2.2E-02	N.S.	1.9E-02	N.S.
	rs1157745	6	1.4E-02	4.4E-02	N.S.	1.2E-02	N.S.	N.S.	5.5E-03	1.9E-02	1.8E-04	1.1E-03
	rs854560	7	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	2.9E-02	N.S.	1.6E-02	N.S.
	rs854565	8	2.1E-03	4.1E-02	3.1E-02	2.1E-02	N.S.	N.S.	1.0E-02	N.S.	1.3E-02	N.S.
	rs705379	9	1.0E-02	N.S.	N.S.	N.S.	N.S.	N.S.	1.9E-03	5.0E-02	8.3E-04	4.4E-02
	rs705381	10	4.1E-02	N.S.	N.S.	9.1E-03	N.S.	N.S.	2.2E-02	N.S.	1.8E-02	N.S.
Intergenic	rs2375001	11	2.4E-02	N.S.	N.S.	4.4E-02	N.S.	N.S.	N.S.	N.S.	3.5E-02	N.S.
	rs1859121	12	N.S.	3.3E-02	4.1E-02	N.S.	N.S.	N.S.	1.5E-02	N.S.	1.4E-02	N.S.
PON3	rs2074352	13	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	1.1E-02	N.S.	5.7E-03	N.S.
	rs3757708	14	N.S.	1.9E-02	4.3E-02	N.S.	N.S.	N.S.	1.1E-02	N.S.	6.3E-03	N.S.
	rs978903	16	3.2E-04	2.1E-02	3.4E-02	2.3E-03	N.S.	N.S.	1.3E-02	N.S.	6.3E-03	N.S.
	rs10487132	17	N.S.	3.8E-02	N.S.	N.S.	N.S.	N.S.	8.3E-03	N.S.	2.3E-03	N.S.
	rs2072200	18	1.1E-02	N.S.	N.S.	3.2E-02	N.S.	N.S.	1.2E-02	N.S.	6.7E-03	N.S.
PON2	rs6954345	19	N.S.	9.7E-03	1.4E-03	N.S.	3.0E-02	1.0E-02	N.S.	N.S.	N.S.	N.S.
	rs3735586	20	5.6E-06	6.5E-04	8.9E-05	5.5E-04	1.6E-02	8.4E-03	1.7E-02	N.S.	1.8E-02	N.S.
	rs10487133	21	7.5E-03	N.S.	N.S.	5.4E-03	N.S.	N.S.	2.9E-02	N.S.	1.3E-02	N.S.
	rs2375005	22	N.S.	1.3E-02	5.1E-03	N.S.	N.S.	N.S.	4.0E-02	N.S.	2.3E-02	N.S.
	rs987539	23	2.8E-03	2.0E-02	1.8E-02	2.2E-02	N.S.	N.S.	2.7E-02	N.S.	1.3E-02	N.S.
	rs6961624	24	1.8E-04	1.8E-02	3.9E-03	4.5E-03	N.S.	4.0E-02	2.5E-02	N.S.	2.2E-02	N.S.
	rs2299263	25	1.9E-04	9.9E-03	2.6E-03	3.6E-03	5.0E-02	3.0E-02	4.2E-02	N.S.	3.1E-02	N.S.
	rs1034809	26	N.S.	2.3E-02	5.7E-03	N.S.	N.S.	N.S.	N.S.	N.S.	4.9E-02	N.S.
	rs2286233	28	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	2.8E-02	N.S.	1.0E-02	N.S.
	rs2299267	29	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	1.7E-02	N.S.	1.2E-02	N.S.

Bold type indicates significant after Bonferroni correction (0.05/52).

Key: N.S., not significant; PA, phenyl acetate; SNP, single nucleotide polymorphism; TBBL, thiobutyl butyrolactone.

lotypes including SNPs spanning the full length of the PON gene cluster were generally more significant than haplotypes comprising SNPs from a smaller region. Thus, we find that lower serum paraoxonase activity is a risk factor for AD and multiple DNA sequence polymorphisms in distinct regions of the PON gene cluster influence serum paraoxonase activity. These results suggest that PON1, PON2, and PON3 may all be involved in AD and that their effects may be synergistic.

Several studies have shown evidence for association of PON with AD risk using DNA markers (Chapuis et al., 2009; Dantoine et al., 2002b; Erlich et al., 2006; He et al., 2006; Janka et al., 2002; Leduc and Poirier, 2008; Scacchi et al., 2003; Shi et al., 2004), but other studies each examining 1 SNP and relatively small samples were negative (Cellini et al., 2006; Pola et al., 2003; Sodeyama et al., 1999; Zuliani et al., 2001). Notably, in an Italian clinic-based sample the PON1 R192Q polymorphism (SNP 3) was not associated with AD (Pola et al., 2003), but carriers of the R allele responded significantly better to treatment with cholinesterase inhibitors (Pola et al., 2005). This finding is consistent with the association findings of L55M (SNP 7) and R192Q with frontal cortex amyloid  $\beta$  level and choline acetyltransferase activity in a group of autopsy-confirmed

Table 5											
Stepwise regression model	including serum	phenyl	l acetate	activity	and	rs3735586	for	Alzheimer'	s di	isease	risk

1 0 0			
Model	OR rs3735586 <sup>a</sup> (95% CI); <i>p</i> -value	OR PA <sup>b</sup> (95% CI); <i>p</i> -value	OR rs3735586 × PA (95% CI); <i>p</i> -value
Age, gender, race <sup>c</sup>	NA	NA	NA
rs3735586	1.28 (1.02–1.59); 0.026	NA	NA
PA	NA	1.22 (1.06–1.40); 0.005	NA
rs3735586, PA	1.19 (0.95–1.50); 0.13	1.19 (1.03-1.38); 0.017	NA
rs3735586, PA, rs3735586 $\times$ PA	0.22 (0.07–0.68); 6.5E-04	2.41 (1.37-4.22); 5.6E-06	0.61 (0.43-0.86); 8.9E-05

Key: CI, confidence interval; OR, odds ratio; PA, phenyl acetate.

<sup>a</sup> Per copy of the A allele.

<sup>b</sup> Per standard deviation unit decrease.

<sup>c</sup> Baseline model covariates included in all models.



Association of SNP rs3735586 with AD

Fig. 5. Interaction of phenyl acetate (PA) activity in serum and rs3735586 genotype on odds of Alzheimer's disease (AD). Odds of AD per unit of PA activity are shown separately for rs3735586 C/T heterozygotes (red curve) and T/T homozygotes (blue curve).

AD patients and age-matched controls (Leduc and Poirier, 2008). A few previous studies examined the association of PON SNPs with the level of serum activity (Dantoine et al., 2002a; Paragh et al., 2002; Wehr et al., 2009), and the results were inconclusive perhaps because of insufficient sample size, consideration of only the phenyl acetate measure which may be a less reliable measure of serum paraoxonase activity than TBBL, or much less comprehensive analysis of variants in the PON gene cluster than the current study.

Findings of association of both serum paraoxonase activity phenotypes with multiple PON1 SNPs is consistent with the observation that coding and regulatory variants in this gene influence the hydrolysis of serum paraoxonase (Brophy et al., 2001b; Deakin et al., 2003; Gaidukov et al., 2006). Our comprehensive SNP analysis also revealed significant association signals in PON2 and PON3, particularly with TBBL. Given the proximity of these genes to one another, these results could be explained most parsimoniously by a single genetic determinant. Alternatively, there may be multiple variants in the PON cluster influencing serum paraoxonase activity. The latter explanation seems more plausible when considering the discontinuous linkage disequilibrium structure of the PON cluster (Erlich et al., 2006) and the results of the haplotype analysis. This hypothesis is also supported by the analyses which showed significant evidence for interaction among SNPs in PON1 with SNPs in PON3 and PON2 to account for variance in activity on TBBL and could explain discrepancies between studies regarding the effect of PON1 (Q192R) (Wheeler et al., 2004) and other PON polymorphisms on various outcomes.

The SNP  $\times$  PA interaction presented in Table 5 suggests that AD risk is not a simple additive function of genotype and PA level. PA has the greatest effect when there are 0 copies of the A allele for rs3735586: for individuals with 0 copies of the A allele, a 1 SD decrease in PA increases odds of disease by a factor of 2.41. For individuals with 1 or 2 copies of the A allele, PA has a more modest effect: a 1 SD decrease in PA increases odds of AD by a factor of 1.47 for the A heterozygotes, and decreases odds of AD by a factor of 0.90 for A/A homozygotes. While the precise mechanism linking paraoxonase to AD pathogenesis is unclear, this interaction suggests that factors other than PON genotype(s) determine PON serum activity which in turn influences AD risk. Another explanation of these findings is that rs3735586 may be tagging different variants with different effects on PA activity level. This is plausible based on haplotype findings (Fig. 3) which show that both the A and T alleles of SNP 20 (also known as rs3735586) are associated with increased PA level and that allele A may also be associated with decreased PA level. Depending on the frequency and dominance pattern of the underlying functional variants in AD subjects and controls, the A/A genotype in linkage disequilibrium (LD) with one variant may be associated with lower PA level in controls whereas the same genotype in LD with another variant may be associated with higher PA level in AD subjects. Also, it is plausible that the PON genotypes may influence AD risk by mechanisms other than PON serum activity levels.

The mechanism by which PON influences AD risk is currently unknown. Low PON1 activity is also associated with increased systemic oxidative stress, and increased risk for cardiovascular disease, type 2 diabetes, and stroke (Aydin et al., 2006; Bhattacharyya et al., 2008; Jarvik et al., 2000; Leviev et al., 2001; Mackness et al., 1991, 1998), all of which are AD risk factors (Rosendorff et al., 2007). A recent study of autopsy-confirmed AD cases showed that the L55M and Q192R variants were associated with  $\beta$ -amyloid levels (p < 0.001), senile plaque accumulation (p < 0.001) 0.001), and choline acetyltransferase activity (p < 0.05) in, respectively, 2 of 2, 5 of 6, and 3 of 6 brain areas (Leduc et al., 2009). However, PON1 does not cross the blood-brain barrier (although an immunoglobulin G [IgG]-PON1 fusion protein has been engineered to penetrate the brain for targeted drug delivery) (Boado et al., 2008) suggesting that paraoxonase is either expressed in brain or may be involved in pathways that can disrupt brain integrity.

Our study has several noteworthy limitations. First, lack of significance for some comparisons within ethnic groups may be due to insufficient sample size for the smaller groups, or differences in lifestyle, behavioral habits, and environmental exposures between ethnic groups. At the same time, the significant results need to be replicated in large independent samples. Nonetheless, the patterns of association appeared the same in all 3 ethnic groups included in our analyses. Second, because serum specimens were collected from nonfasting subjects who provided limited information on medical and medication history, our analyses do not take into account the potential influences of lipid levels and anti-inflammatory medication use on serum paraoxonase activity. Third, the artificial substrates we used to assay serum paraoxonase may not accurately represent in vivo substrates present in brain. Finally, while several of the most significantly associated haplotypes included coding and regulatory variants in PON1, these haplotypes also implicated PON2 intronic SNPs rs3735586 and rs1034809 which were not significant when analyzed individually and are not known to have a biological function.

In summary, our results suggest that deficient serum paraoxonase activity is a significant risk factor for AD and that paraoxonase activity is governed in part by at least 2 distinct variants, one located in the PON1 region and another in PON2. Further studies are needed to confirm the identity of these variants and elucidate their influence on paraoxonase activity and ultimately on AD pathogenesis.

#### **Disclosure statement**

The authors declare that they have no conflicts of interest.

Only persons who gave written informed consent are included in this study. Study protocols were approved by Institutional Review Boards at each recruitment site.

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### Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging. 2010.08.003.

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Supplementary Fig. 1. Linkage disequilibrium (LD) block structure in the PON gene cluster. The block structure was derived from an enlarged sample of 730 Caucasian and 467 African American Alzheimer's disease (AD) cases and controls (Erlich et al., 2006). A scheme of the PON locus is shown, with linkage disequilibrium (LD) maps for African Americans located above and Caucasians below the gene structure. Single nucleotide polymorphisms (SNPs) 5, 15, and 27 were not polymorphic and therefore not included. The predicted functional significance of each SNP is denoted by the symbol color: red = cSNP, blue = intron, orange = 5' untranslated, and gray = intergenic. The measure of LD (D') among all possible pairs of SNPs is shown graphically according to the shade of red where white represents very low D' and dark red represents very high D'. High D' estimates associated with a large confidence interval (most likely due to 1 of the alleles being rare) are denoted by blue squares. Reused with permission.

Supplementary Table 1 Distribution of serum thiobutyl butyrolactone activity according to PON SNP genotype

	Marker	AA freq (%)	AB freq (%)	BB freq (%)	AA mean (SD)	AB mean (SD)	BB mean (SD)
All	rs2237582	218 (37.6)	261 (45.0)	101 (17.4)	20.4 (4.8)	25.0 (5.4)	27.8 (6.0)
	rs2269829	243 (41.6)	251 (43.0)	90 (15.4)	20.7 (5.0)	25.4 (5.5)	27.7 (5.6)
	rs662	102 (17.6)	264 (45.4)	215 (37.0)	27.8 (6.0)	25.2 (5.4)	20.4 (4.9)
	rs2299255	10 (1.7)	132 (22.7)	439 (75.6)	31.2 (2.8)	27.6 (5.0)	22.6 (5.7)
	rs1157745	213 (37.1)	260 (45.3)	101 (17.6)	20.4 (4.9)	25.1 (5.4)	27.8 (6.0)
	rs854560	271 (46.7)	262 (45.2)	47 (8.1)	26.4 (5.5)	22.0 (5.3)	18.5 (5.2)
	rs854565	40 (6.9)	244 (42.4)	292 (50.7)	24.4 (4.8)	23.6 (5.8)	24.1 (6.3)
	rs705379	115 (19.7)	290 (49.6)	180 (30.8)	18.9 (4.9)	23.5 (4.9)	27.4 (6.0)
	rs705381	331 (56.7)	223 (38.2)	30 (5.1)	23.0 (5.9)	24.6 (6.1)	26.6 (5.8)
	rs2375001	339 (58.2)	213 (36.6)	30 (5.2)	22.2 (5.9)	25.6 (5.2)	29.2 (4.4)
	rs1859121	167 (28.6)	298 (51.1)	118 (20.2)	25.4 (5.6)	24.4 (5.9)	20.2 (5.3)
	rs2074352	354 (60.5)	183 (31.3)	48 (8.2)	24.3 (6.4)	22.7 (5.2)	23.7 (5.6)
	rs3757708	163 (27.8)	294 (50.1)	130 (22.1)	25.5 (5.6)	24.4 (5.8)	20.5 (5.6)
	rs978903	120 (20.7)	294 (50.7)	166 (28.6)	20.4 (5.7)	24.2 (5.9)	25.5 (5.6)
	rs10487132	279 (47.6)	239 (40.8)	68 (11.6)	25.8 (5.9)	22.9 (5.4)	19.0 (4.8)
	rs2072200	48 (8.2)	192 (32.7)	348 (59.2)	23.6 (5.7)	22.8 (5.2)	24.4 (6.4)
	rs6954345	37 (6.4)	207 (35.8)	334 (57.8)	28.0 (5.4)	25.9 (5.6)	22.1 (5.7)
	rs3/35586	329 (56.5)	211 (36.3)	42 (7.2)	22.0 (5.6)	25.9 (5.6)	27.6 (5.3)
	rs1048/133	6 (1.0)	137 (23.6)	437 (75.3)	27.8 (2.7)	24.4 (6.1)	23.6 (6.0)
	rs2375005	1/1 (29.5)	291 (50.3)	117 (20.2)	25.4 (5.9)	24.1 (6.1)	20.8 (5.0)
	rs98/539	116(20.0)	286 (49.2)	1/9 (30.8)	20.4 (4.9)	24.1 (6.0)	25.5 (5.8)
	150901024	335 (57.5)	211(30.2)	37 (0.3)	22.1 (5.7)	25.9 (5.0)	28.0 (5.4)
	rs2299263	335 (57.7)	211 (36.3)	35 (6.0)	22.0 (5.7)	25.9 (5.6)	2.08 (5.4)
	rs1034809	03 (11.0)	180(31.5) 127(22.4)	328 (37.4)	20.2 (5.8)	20.1(5.4)	22.0(5.8)
	182200255	439 (73.0)	137(23.4) 196(21.8)	9(1.3)	25.5(5.9)	24.5(0.5)	27.5(5.5)
Couponian	IS2299207	3/1(03.4)	180(31.8) 104(42.5)	28 (4.8)	24.5 (0.0)	22.0 (5.8)	23.0 (0.3)
Caucasian	182237382	209 (40.9)	194 (45.5)	45 (9.0)	20.5(4.8)	24.8(5.0) 24.0(5.1)	28.1(3.0)
	182209629 ro662	219 (46.6) 42 (0.7)	109(42.1) 106(44.1)	41(9.1) 205 (46.2)	20.3 (4.6)	24.9(5.1) 24.9(5.0)	20.2(4.0)
	rs2200255	43(9.7) 10(2.3)	190(44.1) 102(23.0)	203(40.2)	26.0(3.1) 31.2(2.8)	24.9(3.0) 27.3(4.7)	20.4(4.8) 21.7(5.0)
	rs1157745	202(46.1)	102(23.0) 103(44.1)	A3 (0.8)	31.2(2.8) 20.4(4.8)	27.3(4.7) 24.9(5.0)	28 (5.0)
	rs854560	168 (37.8)	234 (52 7)	43(9.5)	26.9(4.7)	24.9(5.0) 21.8(5.2)	18.2(4.7)
	rs854565	30 (6 8)	195(441)	217 (49 1)	23.9(4.4)	21.0(5.2) 22.9(5.3)	13.2(4.7) 23.3(6.0)
	rs705379	98 (21.9)	243 (54 2)	107(23.9)	183(43)	22.9(5.5) 23.4(4.9)	25.5(0.0) 26.9(4.8)
	rs705381	258 (57.6)	170(37.9)	20(45)	222(53)	241(57)	26.2 (5.6)
	rs2375001	258 (57.2)	168 (37.3)	25 (5.5)	21.2(5.3)	25.1 (4.8)	28.9(4.3)
	rs1859121	96 (21.4)	245 (54.7)	107 (23.9)	25.1 (5.2)	23.7 (5.3)	19.9 (5.2)
	rs2074352	278 (61.8)	150 (33.3)	22 (4.9)	23.5 (5.9)	22.4 (5.1)	22.8 (5.0)
	rs3757708	90 (20.0)	246 (54.5)	115 (25.5)	25.2 (5.2)	23.7 (5.4)	20.0 (5.1)
	rs978903	107 (23.9)	246 (55.0)	94 (21.0)	19.8 (5.2)	23.7 (5.4)	25.3 (5.1)
	rs10487132	159 (35.3)	224 (49.8)	67 (14.9)	25.3 (5.0)	22.8 (5.4)	19.0 (4.8)
	rs2072200	24 (5.3)	156 (34.7)	270 (60.0)	22.5 (5.0)	22.4 (5.0)	23.5 (6.0)
	rs6954345	26 (5.9)	149 (33.7)	267 (60.4)	29.2 (4.3)	25.4 (5.0)	21.3 (5.2)
	rs3735586	269 (60.2)	151 (33.8)	27 (6.0)	21.3 (5.1)	25.4 (5.0)	28.5 (4.7)
	rs10487133	5 (1.1)	104 (23.4)	336 (75.5)	27.3 (2.7)	23.7 (5.2)	22.9 (5.7)
	rs2375005	92 (20.7)	244 (54.8)	109 (24.5)	25.3 (5.4)	23.5 (5.5)	20.6 (5.0)
	rs987539	109 (24.4)	243 (54.5)	94 (21.1)	20.3 (4.9)	23.5 (5.5)	25.3 (5.2)
	rs6961624	267 (59.9)	153 (34.3)	26 (5.8)	21.3 (5.1)	25.4 (4.9)	29.2 (4.3)
	rs2299263	269 (60.0)	155 (34.6)	24 (5.4)	21.2 (5.1)	25.3 (5.0)	29.3 (4.3)
	rs1034809	40 (9.0)	141 (31.8)	262 (59.1)	27.6 (4.7)	25.3 (5.1)	21.2 (5.1)
	rs2286233	342 (75.8)	104 (23.1)	5 (1.1)	22.9 (5.7)	23.6 (5.2)	27.3 (2.7)
	rs2299267	287 (63.9)	143 (31.8)	19 (4.2)	23.6 (5.6)	22.2 (5.3)	21.9 (6.3)
African American	rs2237582	5 (6.4)	42 (53.8)	31 (39.7)	22.5 (4.2)	26.3 (7.0)	30.5 (5.4)
	rs2269829	20 (25.0)	36 (45.0)	24 (30)	23.5 (6.4)	28.7 (6.7)	29.9 (5.1)
	rs662	32 (39.5)	43 (53.1)	6 (7.4)	30.5 (5.3)	26.4 (7.0)	22.7 (3.8)
	rs2299255	18 (22.2)	63 (77.8)	0 (0.0)	31.1 (4.5)	26.8 (6.8)	
	rs1157745	7 (8.8)	42 (52.5)	31 (38.8)	23.5 (4.1)	26.5 (7.1)	30.5 (5.4)
	rs854560	61 (75.3)	17 (21.0)	3 (3.7)	28.8 (6.3)	24.2 (6.6)	25.3 (5.9)
	rs854565	10 (12.8)	31 (39.7)	37 (47.4)	25.9 (5.9)	27.2 (7.3)	29.1 (5.9)
	rs705379	1 (1.2)	22 (27.2)	58 (71.6)	11.3 (.)	25.4 (4.1)	28.9 (6.8)
	rs705381	26 (32.5)	44 (55.0)	10 (12.5)	28.9 (6.0)	27.1 (7.1)	27.3 (6.4)
	rs2375001	43 (55.1)	30 (38.5)	5 (6.4)	26.3 (7.4)	29.4 (5.2)	30.5 (5.2)
	rs1859121	25 (31.3)	44 (55.0)	11 (13.8)	29.9 (4.9)	27.6 (7.1)	23.1 (5.8)

(continued on next page)

Supplementary Table 1	
(continued)	

	Marker	AA freq (%)	AB freq (%)	BB freq (%)	AA mean (SD)	AB mean (SD)	BB mean (SD)
	rs2074352	67 (83.8)	13 (16.3)	0 (0.0)	28.1 (6.7)	25.6 (5.8)	
	rs3757708	26 (32.5)	39 (48.8)	15 (18.8)	29.8 (4.9)	28.0 (6.4)	23.8 (8.3)
	rs978903	13 (16.3)	39 (48.8)	28 (35.0)	25.4 (7.6)	27.0 (7.0)	29.6 (4.9)
	rs10487132	64 (80.0)	15 (18.8)	1 (1.3)	28.7 (6.6)	24.6 (5.6)	19.3 (.)
	rs2072200	13 (15.9)	69 (84.1)	0 (0.0)	25.6 (5.8)	28.1 (6.6)	
	rs6954345	5 (6.3)	39 (48.8)	36 (45.0)	31.2 (4.9)	29.1 (6.0)	25.8 (7.0)
	rs3735586	30 (37.5)	41 (51.3)	9 (11.3)	26.3 (6.7)	28.7 (6.3)	30.0 (4.2)
	rs10487133	1 (1.3)	30 (38.0)	48 (60.8)	30.3 (.)	26.6 (7.9)	28.4 (5.8)
	rs2375005	27 (34.2)	44 (55.7)	8 (10.1)	29.5 (5.0)	27.6 (7.5)	22.8 (4.5)
	rs987539	7 (8.8)	40 (50.0)	33 (41.3)	22.3 (4.6)	27.5 (7.4)	29.2 (5.4)
	rs6961624	37 (45.7)	39 (48.1)	5 (6.2)	25.9 (6.9)	29.1 (6.0)	31.2 (4.9)
	rs2299263	36 (45.6)	38 (48.1)	5 (6.3)	25.8 (7.0)	29.2 (6.1)	31.2 (4.9)
	rs1034809	6 (7.5)	33 (41.3)	41 (51.3)	29.5 (5.2)	29.3 (5.3)	26.1 (7.4)
	rs2286233	45 (57.0)	30 (38.0)	4 (5.1)	28.3 (5.1)	26.7 (8.7)	27.3 (4.5)
	rs2299267	64 (80.0)	15 (18.8)	1 (1.3)	28.2 (6.5)	26.3 (7.2)	28.6 (.)
Asian American	rs2237582	4 (7.1)	25 (44.6)	27 (48.2)	15.8 (6.6)	24.7 (5.4)	24.2 (6.4)
	rs2269829	4 (7.3)	26 (47.3)	25 (45.5)	15.8 (6.6)	24.5 (5.4)	24.8 (6.2)
	rs662	27 (48.2)	25 (44.6)	4 (7.1)	24.2 (6.4)	24.7 (5.4)	15.8 (6.6)
	rs2299255	12 (21.4)	44 (78.6)	0 (0.0)	25.8 (6.1)	23.3 (6.3)	× /
	rs1157745	4 (7.1)	25 (44.6)	27 (48.2)	15.8 (6.6)	24.7 (5.4)	24.2 (6.4)
	rs854560	42 (76.4)	11 (20.0)	2 (3.6)	24.8 (6.1)	21.6 (3.9)	13.0 (9.7)
	rs854565	18 (32.1)	38 (67.9)	0 (0.0)	25.0 (6.1)	23.3 (6.4)	× /
	rs705379	16 (28.6)	25 (44.6)	15 (26.8)	23.0 (6.2)	23.7 (4.8)	25.1 (8.5)
	rs705381	47 (83.9)	9 (16.1)	0 (0.0)	24.0 (6.6)	23.2 (4.8)	× /
	rs2375001	38 (71.7)	15 (28.3)	0 (0.0)	24.1 (6.1)	23.1 (6.4)	
	rs1859121	46 (83.6)	9 (16.4)	0 (0.0)	23.3 (5.6)	25.7 (9.1)	
	rs2074352	9 (16.4)	20 (36.4)	26 (47.3)	22.1 (8.8)	23.6 (5.4)	24.4 (6.0)
	rs3757708	47 (83.9)	9 (16.1)	0 (0.0)	23.5 (5.7)	25.7 (9.1)	× /
	rs978903	9 (17.0)	44 (83.0)	0 (0.0)	25.7 (9.1)	23.4 (5.6)	
	rs10487132	56 (100)	0 (0.0)	0 (0.0)	23.9 (6.3)		
	rs2072200	24 (42.9)	23 (41.1)	9 (16.1)	24.6 (6.2)	24.1 (5.4)	21.4 (8.5)
	rs6954345	6 (10.7)	19 (33.9)	31 (55.4)	20.1 (1.9)	23.7 (7.3)	24.7 (6.1)
	rs3735586	30 (54.5)	19 (34.5)	6 (10.9)	24.6 (6.2)	23.7 (7.3)	20.1 (1.9)
	rs10487133	3 (5.4)	53 (94.6)	0 (0.0)	27.9 (8.7)	23.6 (6.2)	× /
	rs2375005	52 (94.5)	3 (5.5)	0 (0.0)	23.6 (6.2)	27.9 (8.7)	
	rs987539	3 (5.5)	52 (94.5)	0 (0.0)	27.9 (8.7)	23.6 (6.2)	
	rs6961624	31 (55.4)	19 (33.9)	6 (10.7)	24.7 (6.1)	23.7 (7.3)	20.1 (1.9)
	rs2299263	30 (55.6)	18 (33.3)	6 (11.1)	24.7 (6.1)	24.2 (7.1)	20.1 (1.9)
	rs1034809	17 (35.4)	6 (12.5)	25 (52.1)	21.6 (5.9)	25.0 (8.1)	24.2 (6.2)
	rs2286233	52 (94.5)	3 (5.5)	0 (0.0)	23.6 (6.2)	27.9 (8.7)	= ()
	rs2299267	20 (35.7)	28 (50.0)	8 (14.3)	25.3 (5.5)	22.6 (6.8)	24.9 (6.3)

Alleles are ordered alphabetically (i.e., A, C, G, T). Key: freq, frequency.

Supplementary Table 2 Distribution of serum phenyl acetate activity according to PON SNP genotype

	Marker	AA freq (%)	AB freq (%)	BB freq (%)	AA mean (SD)	AB mean (SD)	BB mean (SD)
All	rs705379	115 (19.7)	289 (49.5)	180 (30.8)	102.3 (23.2)	127.7 (30.4)	155.6 (48.4)
	rs2299263	334 (57.7)	210 (36.3)	35 (6.0)	122.4 (36.7)	143.3 (43.5)	144.1 (34.5)
	rs2299255	10 (1.7)	132 (22.8)	438 (75.5)	168.8 (32.6)	145.5 (37.3)	126.4 (40.0)
	rs2286233	437 (75.0)	137 (23.5)	9 (1.5)	128.9 (39.1)	135.4 (43.1)	166.2 (41.1)
	rs2237582	218 (37.7)	260 (44.9)	101 (17.4)	117.8 (31.1)	137.3 (39.8)	144.1 (51.1)
	rs2269829	242 (41.6)	250 (43.0)	90 (15.5)	118.7 (31.6)	138.8 (40.4)	145.2 (51.1)
	rs2375001	338 (58.3)	212 (36.6)	30 (5.2)	122.9 (39.2)	142.9 (40.0)	145.6 (30.7)
	rs2074352	354 (60.7)	182 (31.2)	47 (8.1)	135.1 (44.0)	126.8 (33.6)	121.7 (33.4)
	rs3757708	161 (27.5)	294 (50.3)	130 (22.2)	136.7 (39.8)	136.5 (41.3)	113.1 (33.8)
	rs10487132	277 (47.4)	239 (40.9)	68 (11.6)	141.1 (44.0)	127.9 (34.4)	104.5 (28.5)
	rs2072200	47 (8.0)	191 (32.6)	348 (59.4)	122.6 (34.4)	125.9 (33.5)	135.4 (44.2)
	rs3735586	328 (56.6)	210 (36.2)	42 (7.2)	122.5 (36.4)	142.1 (43.0)	147.1 (40.0)
	rs2375005	169 (29.3)	291 (50.4)	117 (20.3)	139.4 (45.6)	132.6 (38.1)	117.2 (34.8)
	rs1034809	63 (11.1)	179 (31.4)	328 (57.5)	137.1 (30.7)	145.7 (45.3)	122.2 (37.0)
	rs2299267	3/1 (63.6)	185 (31.7)	27 (4.6)	134.4 (42.3)	126.1 (35.5)	123.3 (33.7)
	rs98/539	116 (20.0)	286 (49.3)	1/8 (30.7)	115.3 (33.9)	132.2 (37.6)	139.6 (45.3)
	rs6954345	37 (6.4)	206 (35.8)	333 (57.8)	143.8 (33.7)	143.0 (43.8)	122.4 (36.7)
	rs9/8903	120 (20.8)	294 (50.9)	164 (28.4)	113.0 (34.3)	135.7 (41.5)	136.7 (39.3)
	rs/05381	330 (56.7)	222 (38.1)	30 (5.2)	123.9 (36.0)	139.0 (43.6)	149.5 (46.9)
	rs854565	40 (7.0)	243 (42.3)	292 (50.8)	137.9 (32.4)	136.5 (44.6)	126.7 (37.6)
	rs115//45	212 (37.1)	259 (45.3)	101 (17.7)	117.8 (31.2)	137.2 (39.5)	143.5 (51.1)
	rs1048/133	6 (1.0)	137 (23.7)	435 (75.3)	155.8 (38.1)	135.2 (39.9)	129.7 (40.5)
	rs1859121	165 (28.4)	298 (51.3)	118 (20.3)	136.1 (39.5)	136.0 (41.6)	112.3 (32.6)
	rs662	102 (17.6)	263 (45.4)	214 (37.0)	143.8 (51.0)	137.4 (39.8)	117.7 (31.5)
	rs6961624	334 (57.5)	210 (36.1)	37 (6.4)	122.7 (36.8)	143.3 (43.6)	143.8 (33.7)
а ·	rs854560	2/0 (46.7)	261 (45.2)	4/(8.1)	145.1 (44.1)	121.4 (32.7)	104.1 (27.5)
Caucasian	rs/053/9	98 (21.9)	242 (54.1)	107(23.9)	101.8 (22.3)	127.2 (30.5)	151.9 (44.8)
	rs2299203	208 (00.1)	154 (54.5)	24 (5.4)	119.0 (33.7)	138.9 (38.9)	147.2 (34.0)
	IS2299255	10(2.3)	102 (23.0)	551 (74.7)	108.8 (32.0)	142.4 (35.9)	122.0(35.4) 142.7(26.0)
	182200233	340(73.7)	104(25.2) 102(42.4)	3(1.1)	123.8(37.0) 117.5(21.0)	132.3(33.3) 122.8(34.0)	143.7(20.9) 150.0(56.0)
	182237382	209(47)	193(43.4) 198(42.1)	43 (9.7)	117.3(31.0) 117.0(20.8)	132.6(34.0) 122.6(24.4)	150.9(50.9)
	182209629	210 (40.0)	160(42.1) 167(27.2)	41(9.2)	117.9(30.6) 110.2(37.0)	133.0(34.4) 127.4(22.7)	131.9(37.4) 145.2(22.5)
	rs2074352	237(37.2) 278(62.1)	107(37.2) 140(33.3)	23(3.0) 21(4.7)	119.3(37.9) 120.2(30.4)	137.4(32.7) 125.7(33.3)	143.2(32.3) 120.7(30.3)
	rs3757708	278 (02.1)	246(54.8)	115 (25.6)	129.2(39.4) 1341(32.2)	123.7(33.3) 132.0(38.0)	120.7(30.3) 1114(314)
	rs10/87132	157 (35.0)	240(54.8) 224(50.0)	67 (15.0)	134.1(32.2) 137.6(39.0)	127.7 (34.6)	104.5(28.7)
	rs2072200	23 (5 1)	15 (34.6)	270 (60 3)	120.6 (31.9)	127.7 (34.0) 125.0 (33.2)	104.3(20.7) 129.4(39.7)
	rs3735586	268 (60 2)	15(33.7)	270 (00.5)	119 3 (33 8)	129.0(39.2) 139.0(39.2)	125.7(33.3) 145.7(33.3)
	rs2375005	90 (20 3)	24(551)	109 (24.6)	137 8 (43 0)	129.2 (34.8)	145.7(35.5) 116.2 (34.0)
	rs1034809	40 (9.0)	14(31.7)	262 (59.3)	141.1(30.8)	139.2 (40.1)	110.2(31.0) 119.0(33.9)
	rs2299267	287 (64 2)	14 (31.8)	18 (4 0)	129.8 (39.2)	123.8 (30.6)	119.8 (34.3)
	rs987539	109 (24.5)	243 (54.6)	93 (20.9)	114.1 (32.8)	129.8 (35.0)	137.2 (42.3)
	rs6954345	26 (5.9)	148 (33.6)	266 (60.5)	146.6 (33.6)	138.9 (39.2)	119.1 (33.7)
	rs978903	107 (24.0)	246 (55.3)	92 (20.7)	110.4 (32.0)	132.5 (38.9)	134.1 (31.9)
	rs705381	257 (57.6)	169 (37.9)	20 (4.5)	121.2 (33.9)	135.1 (39.3)	138.3 (44.0)
	rs854565	30 (6.8)	194 (44.0	217 (49.2)	133.8 (30.5)	130.9 (39.8)	124.1 (35.5)
	rs1157745	201 (46.1)	192 (44.0)	43 (9.9)	117.2 (31.1)	132.6 (33.5)	149.5 (57.1)
	rs10487133	5(1.1)	104 (23.5)	334 (75.4)	143.7 (26.9)	132.7 (35.3)	125.7 (37.5)
	rs1859121	94 (21.1)	245 (54.9)	107 (24.0)	133.2 (32.3)	132.6 (38.8)	110.7 (31.8)
	rs662	43 (9.7)	195 (44.1)	204 (46.2)	149.5 (57.1)	133.2 (34.2)	117.3 (31.5)
	rs6961624	266 (59.9)	152 (34.2)	26 (5.9)	119.3 (33.9)	139.4 (38.9)	146.6 (33.6)
	rs854560	167 (37.8)	233 (52.7)	42 (9.5)	142.1 (39.4)	120.6 (32.6)	103.3 (28.2)
African American	rs705379	98 (21.9)	242 (54.1)	107 (23.9)	101.8 (22.3)	127.2 (30.5)	151.9 (44.8)
	rs2299263	268 (60.1)	154 (34.5)	24 (5.4)	119.0 (33.7)	138.9 (38.9)	147.2 (34.6)
	rs2299255	10 (2.3)	102 (23.0)	331 (74.7)	168.8 (32.6)	142.4 (35.9)	122.0 (35.4)
	rs2286233	340 (75.7)	104 (23.2)	5 (1.1)	125.8 (37.6)	132.3 (35.3)	143.7 (26.9)
	rs2237582	209 (47)	193 (43.4)	43 (9.7)	117.5 (31.0)	132.8 (34.0)	150.9 (56.9)
	rs2269829	218 (48.8)	188 (42.1)	41 (9.2)	117.9 (30.8)	133.6 (34.4)	151.9 (57.4)
	rs2375001	257 (57.2)	167 (37.2)	25 (5.6)	119.3 (37.9)	137.4 (32.7)	145.2 (32.5)
	rs2074352	278 (62.1)	149 (33.3)	21 (4.7)	129.2 (39.4)	125.7 (33.3)	120.7 (30.3)
	rs3757708	88 (19.6)	246 (54.8)	115 (25.6)	134.1 (32.2)	132.9 (38.9)	111.4 (31.4)
	rs10487132	157 (35.0)	224 (50.0)	67 (15.0)	137.6 (39.0)	127.7 (34.6)	104.5 (28.7)
	rs2072200	23 (5.1)	155 (34.6)	270 (60.3)	120.6 (31.9)	125.0 (33.2)	129.4 (39.7)
	rs3735586	268 (60.2)	150 (33.7)	27 (6.1)	119.3 (33.8)	139.0 (39.2)	145.7 (33.3)

(continued on next page)

Supplementary	Table 2
(continued)	

	Marker	AA freq (%)	AB freq (%)	BB freq (%)	AA mean (SD)	AB mean (SD)	BB mean (SD)
	rs2375005	90 (20.3)	244 (55.1)	109 (24.6)	137.8 (43.0)	129.2 (34.8)	116.2 (34.0)
	rs1034809	40 (9.0)	140 (31.7)	262 (59.3)	141.1 (30.8)	139.2 (40.1)	119.0 (33.9)
	rs2299267	287 (64.2)	142 (31.8)	18 (4.0)	129.8 (39.2)	123.8 (30.6)	119.8 (34.3)
	rs987539	109 (24.5)	243 (54.6)	93 (20.9)	114.1 (32.8)	129.8 (35.0)	137.2 (42.3)
	rs6954345	26 (5.9)	148 (33.6)	266 (60.5)	146.6 (33.6)	138.9 (39.2)	119.1 (33.7)
	rs978903	107 (24.0)	246 (55.3)	92 (20.7)	110.4 (32.0)	132.5 (38.9)	134.1 (31.9)
	rs705381	257 (57.6)	169 (37.9)	20 (4.5)	121.2 (33.9)	135.1 (39.3)	138.3 (44.0)
	rs854565	30 (6.8)	194 (44.0)	217 (49.2)	133.8 (30.5)	130.9 (39.8)	124.1 (35.5)
	rs1157745	201 (46.1)	192 (44.0)	43 (9.9)	117.2 (31.1)	132.6 (33.5)	149.5 (57.1)
	rs10487133	5(1.1)	104 (23.5)	334 (75.4)	143.7 (26.9)	132.7 (35.3)	125.7 (37.5)
	rs1859121	94 (21.1)	245 (54.9)	107 (24.0)	133.2 (32.3)	132.6 (38.8)	110.7 (31.8)
	rs662	43 (9.7)	195 (44.1)	204 (46.2)	149.5 (57.1)	133.2 (34.2)	117.3 (31.5)
	rs6961624	266 (59.9)	152 (34.2)	26 (5.9)	119.3 (33.9)	139.4 (38.9)	146.6 (33.6)
	rs854560	167 (37.8)	233 (52.7)	42 (9.5)	142.1 (39.4)	120.6 (32.6)	103.3 (28.2)
Asian American	rs2237582	4 (7.1)	25 (44.6)	27 (48.2)	103.6 (13.5)	144.3 (43.2)	117.0 (36.1)
	rs2269829	4 (7.3)	26 (47.3)	25 (45.5)	103.6 (13.5)	142.4 (43.5)	119.2 (36.7)
	rs662	27 (48.2)	25 (44.6)	4 (7.1)	117.0 (36.1)	144.3 (43.2)	103.6 (13.5)
	rs2299255	12 (21.4)	44 (78.6)	0 (0.0)	147.7 (41.2)	123.0 (39.5)	
	rs1157745	4 (7.1)	25 (44.6)	27 (48.2)	103.6 (13.5)	144.3 (43.2)	117.0 (36.1)
	rs854560	42 (76.4)	11 (20.0)	2 (3.6)	131.5 (44.3)	123.4 (27.7)	106.0 (22.9)
	rs854565	18 (32.1)	38 (67.9)	0 (0.0)	146.7 (49.2)	119.5 (33.5)	
	rs705379	16 (28.6)	25 (44.6)	15 (26.8)	107.0 (28.4)	131.9 (32.8)	144.8 (54.8)
	rs705381	47 (83.9)	9 (16.1)	0 (0.0)	127.7 (41.9)	131.3 (36.8)	
	rs2375001	38 (71.7)	15 (28.3)	0 (0.0)	127.7 (31.8)	135.4 (56.5)	
	rs1859121	46 (83.6)	9 (16.4)	0 (0.0)	123.9 (34.7)	154.8 (60.1)	
	rs2074352	9 (16.4)	20 (36.4)	26 (47.3)	139.7 (64.5)	132.3 (33.7)	122.6 (36.3)
	rs3757708	47 (83.9)	9 (16.1)	0 (0.0)	123.2 (34.7)	154.8 (60.1)	
	rs978903	9 (17.0)	44 (83)	0 (0.0)	154.8 (60.1)	122.7 (33.9)	
	rs10487132	56 (100)	0 (0.0)	0 (0.0)	128.3 (40.8)		
	rs2072200	24 (42.9)	23 (41.1)	9 (16.1)	124.5 (37.2)	128.1 (34.0)	138.8 (64.3)
	rs6954345	6 (10.7)	19 (33.9)	31 (55.4)	115.8 (21.9)	132.3 (52.1)	128.2 (36.1)
	rs3735586	30 (54.5)	19 (34.5)	6 (10.9)	127.6 (36.6)	132.3 (52.1)	115.8 (21.9)
	rs10487133	3 (5.4)	53 (94.6)	0 (0.0)	167 (110.2)	126.1 (34.7)	
	rs2375005	52 (94.5)	3 (5.5)	0 (0.0)	125.4 (34.7)	167 (110.2)	
	rs987539	3 (5.5)	52 (94.5)	0 (0.0)	167 (110.2)	124.8 (33.8)	
	rs6961624	31 (55.4)	19 (33.9)	6 (10.7)	128.2 (36.1)	132.3 (52.1)	115.8 (21.9)
	rs2299263	30 (55.6)	18 (33.3)	6 (11.1)	129.3 (36.2)	134.8 (52.4)	115.8 (21.9)
	rs1034809	17 (35.4)	6 (12.5)	25 (52.1)	125.4 (31.2)	149.5 (78.9)	123.8 (35.7)
	rs2286233	52 (94.5)	3 (5.5)	0 (0.0)	124.8 (33.8)	167 (110.2)	
	rs2299267	20 (35.7)	28 (50.0)	8 (14.3)	135.3 (47.7)	123.7 (38.1)	126.4 (32.9)

Alleles are ordered alphabetically (i.e., A, C, G, T). Key: freq, frequency; SNP, single nucleotide polymorphism.





Supplementary Fig. 2. Distribution of serum paraoxonase activity by ethnic group in AD cases and controls. Each box demarcates the 25–75 percentiles with the median shown therein. Whiskers extend to the highest and lowest data points within 1.5 IQR of the upper and lower quartiles, respectively. Outliers are shown as dots. (A) Thiobutyl butyrolactone; (B) phenyl acetate. See the methods section for explanation of activity units.