Published Ahead of Print on July 5, 2006 as 10.1212/01.wnl.0000227187.52002.88

Paraoxonase cluster polymorphisms are associated with sporadic ALS

M. Saeed, MD; N. Siddique, RN, MSN; W.-Y. Hung, PhD; E. Usacheva, PhD; E. Liu, MD; R.L. Sufit, MD; S.L. Heller, MD; J.L. Haines, PhD; M. Pericak-Vance, PhD; and T. Siddique, MD

Abstract—*Background:* Paraoxonases (PONs) are involved in the detoxification of organophosphate pesticides and chemical nerve agents. Due to a reported possible twofold increased risk of ALS in Gulf War veterans and the associations of *PON1* polymorphisms with the neurologic symptom complex of the Gulf War syndrome, the authors investigated the association between sporadic ALS (SALS) and *PON* gene cluster variants in a large North American Caucasian family–based and case-control cohort (N = 1,891). *Methods:* Clinically definite and probable ALS was diagnosed according to the revised El Escorial criteria, exclusion of family history of ALS, and *SOD1* mutation analysis. Single nucleotide polymorphism (SNP) genotyping was done using *Taq*Man assays on ABI7900HT. Data were analyzed using SPSS, Haploview, FBAT, and THESIAS. *Results:* A haploblock of high linkage disequilibrium (LD) spanning *PON2* and *PON3* was associated with SALS. The SNPs rs10487132 and rs11981433 were in strong LD and associated with SALS in the trio (parents-affected child triad) model. The association of rs10487132 was replicated in 450 nuclear pedigrees comprising trios and discordant sibpairs. No association was found in case-control models, and their haplostructure was different from that of the trios with overall reduced LD. Resequencing identified an intronic variant (rs17876088) that differentiated between detrimental and protective SALS haplotypes. *Conclusion:* This study demonstrates evidence of significant association of variants in the Paraoxonase gene cluster with sporadic ALS and is compatible with the hypothesis that environmental toxicity in a susceptible host may precipitate ALS.

NEUROLOGY 2006;67:1–1

Although *SOD1* accounts for 20% of familial ALS (2% of all ALS), the etiology of sporadic ALS (SALS) is unknown.[1] SALS is hypothesized to be a complex disorder in which disease is modulated by variations in multiple genetic loci interacting with each other and environmental exposures.

The human paraoxonases (PONs) are detoxifying enzymes involved in the metabolism of a variety of drugs, oxidized products of arachidonic acid, organophosphates, neurotoxins, and arylesters.[2,3] The genetic locus encoding *PON* spans 140 kb on chromosome 7q21.3 and contains the three members of the *PON* gene family.[4] PON1 and PON3 are expressed primarily in liver, while PON2 is expressed widely including the brain and liver.[2,5,6] Although PON activity remains stable over time in a given individual; there is 10- to 40-fold interindividual variability.[7-9] There are indications that this variability may be under genetic control.[8-10]

Due to a reported possible twofold increased risk of ALS in Gulf War veterans,[11-14] and the associations of *PON1* polymorphisms with the neurologic symptom complex of the Gulf War syndrome,[15-20] we investigated the association between SALS and *PON* gene cluster variants in a large North American Caucasian family–based and case-control cohort (N = 1,891).

Methods. *Subjects.* The data set consisted of 2,009 subjects with 6% non-Caucasians who were removed at the analysis phase.

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the August 22 issue to find the title link for this article.

Editorial, see page XXX
See also page XXX

This article was previously published in electronic format as an Expedited E-Pub at www.neurology.org.

From the Davee Department of Neurology and Clinical Neurosciences (M.S., N.S., W.-Y.H., E.U., E.L., R.L.S., S.L.H.), Feinberg School of Medicine, Northwestern University, Chicago, IL; Center for Human Genetics Research (J.L.H.), Vanderbilt University Medical Center, Nashville, TN; Center for Human Genetics (M.P.-V.), Duke University Medical Center, Durham, NC; and Department of Cell and Molecular Biology (T.S.), Northwestern University Institute of Neuroscience, Chicago, IL.

Supported by the National Institute of Neurological Disorders and Stroke (NS050641, NS046535), the Les Turner ALS Foundation, the V. E. Schaff ALS Research Fund, the H. Post Research Professorship, the Wenske Foundation, the Falk Medical Research Trust, Abbott Labs Duane and Susan Burnham Professorship, and the David C. Asselin MD Memorial Fund.

Disclosure: The authors report no conflicts of interest

Received January 23, 2006. Accepted in final form April 25, 2006.

Address correspondence and reprint requests to Dr. Teepu Siddique, Davee Department of Neurology and Clinical Neurosciences and Department of Cell and Molecular Biology, Northwestern University Feinberg School of Medicine, Tarry Building, Room 13-715, 303 East Chicago Avenue, Chicago IL 60611; e-mail: t-siddique@northwestern.edu

Copyright © 2006 by AAN Enterprises, Inc.

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Thus, the Caucasian cohort consisted of 1,891 subjects: 203 unaffected parents-affected child triads (trios), 247 discordant sibpairs (DSPs), 284 cases, and 504 spousal/community control subjects. The subject and family ascertainment was conducted through clinics in North America specializing in ALS. All patients diagnosed with SALS were examined by a neurologist and met the revised El Escorial criteria for the diagnosis of clinically definite or probable ALS.[21] Clinical information, including site of onset (bulbar/upper motor neuron onset and spinal/lower motor neuron onset) and age at onset of the patient (defined as the time at which the first significant weakness occurred), was obtained at the time of examination. Informed consent was obtained for the study from all participants. The data were collected according to protocols approved by our Institutional Review Board and met Health Insurance Portability and Accountability Act standards of confidentiality and disclosure.

Our strategy was to map the *PON* cluster first with a few well-spaced single nucleotide polymorphisms (SNPs) in a case-control and family-based testing cohort and identify the association signal with genotype and haplotype analysis. The same cohort was then densely fine-mapped to accurately localize the signal and the association was validated by replication in our entire cohort of 1,891 subjects (case-control and family-based models). We then resequenced the genes in the associated region to identify functional variants.

Genotyping. Genomic DNA was extracted from whole blood according to established protocols.[22] High-throughput SNP genotyping was performed using *Taq*man assay in a 384-well format on the ABI prism 7900HT sequence detection system (Applied Biosystems). In each chamber of the 384-well plate, 10 ng of genomic DNA was used. PCR primers and probes were obtained from Applied Biosystems assays-on-demand service. Reactions were run in 5-μL volumes using *Taq*man Universal PCR master mix, primers, and probes. The amplification protocol was 95C for 10 minutes followed by 40 cycles of 95C for 5 seconds and 60C for 1 minute. Genotyping data were obtained using the ABI-PRISM sequence detection system (SDS) software version 2.1.

Statistical analysis. Data were analyzed using the SPSS (Statistical Package for Social Sciences) Software Version 13.0 for Windows (Gorinchem, The Netherlands). Case-control genotype associations were assessed by χ^2 analyses. Association with subphenotypes, such as site and age at onset, was explored using the same approach. Kaplan-Meier curves were constructed for age-at-onset data. Estimations of departures from Hardy-Weinberg equilibrium (HWE) were calculated by χ^2 test. We considered nominal p values <0.05 significant for the preliminary screen and fine-mapping, aimed to localize the association signal. For the replication phase, we applied false discovery rate (FDR) as correction for multiple testing.[23]

The transmission/disequilibrium test (TDT), as detailed in the program FBAT version 1.5.5, was used for testing associations in the trio and DSP groups as well as their combined analysis.[24] The test uses a modified χ^2 statistic to determine overtransmission of an allele to an affected offspring. Haplotype frequencies and association statistics were estimated using an expectation maximization algorithm incorporated in FBAT (HBAT routine) for the trios, DSPs, and all nuclear pedigrees combined. THESIAS version 2.0 was used to estimate haplotype statistics for case-control groups.[25] Haploview was used to calculate linkage disequilibrium (LD) statistics.[26] The Tagger routine incorporated in Haploview was used to select tagging SNPs for haplotype analysis.[26] Redundant SNPs with $r^2 \geq 0.8$ were removed before haplotype analysis.

To identify SNPs for replication in the entire cohort, SNPs that, at the fine-mapping stage, independently demonstrated association or were part of an associated haplotype were considered. Two-locus haplotype analysis was done for all SNPs in the trio and case-control models to define the LD pattern for the SNPs and the strength of the association of their haplotypes. Associated SNPs in high LD ($r^2 = 0.60$) with each other were taken to represent a single signal, and only one of the SNPs, based on the lowest p value (genotype and two-locus haplotype), was chosen for replication. Furthermore, sliding window analysis was carried out to identify the most significantly associated haploblocks. SNPs comprising these haploblocks were analyzed for genotype and two-locus haplotype significance, and only those SNPs that demonstrated association were deemed valuable for replication.

HelixTree is propriety software for association studies of large data sets.[27] Haplotype estimation is implemented in HelixTree using two independent methods (estimation maximization and composite haplotype). Both methods have their own advantages, but the expectation-maximization algorithm is more robust in the absence of HWE. HelixTree is able to assign probability scores to every sample for each haplotype estimated. This unique feature allowed us to select samples for resequencing based on their estimated haplotypes.

Table 1 *Clinical characteristics of SALS patients and control subjects*

	Age (mean ± SD)	Gender (M/F)	Total (no.)
Trio probands	39.8 ± 9.5	136/67	203
DSP probands	53.3 ± 11.9	135/112	247
Sporadic cases	55.6 ± 13.6	172/112	284
Controls	60.5 ± 14.8	199/305	504
Bulbar onset*	53.7 ± 14.7	73/62	135
Spinal onset*	49.2 ± 13.2	318/191	509
UL onset*	46.7 ± 13.4	196/89	285
LL onset*	52.3 ± 12.3	111/98	209
EOALS	33.8 ± 5.2	156/71	227
LOALS	56.4 ± 10.4	286/221	507

* Complete subphenotype data were not available on all sporadic ALS patients.

SALS = sporadic ALS; DSP = discordant sib pairs; UL = upper limb; LL = lower limb; EOALS = early-onset ALS; LOALS = late-onset ALS.

Results. The clinical characteristics of our patients and controls are described (table 1). We further classified our patients based on subphenotypes such as age at onset and site of onset (bulbar, spinal, upper limb, and lower limb). Arbitrarily taking the age at onset of 40 years as a cutoff, we divided SALS probands into an early-onset subgroup with age 40 years and younger and a late-onset group with age older than 40 years. This cutoff has been used previously to show a genetic modifier effect.[28]

Preliminary screen. We screened 186 trios and 186 controls both of Caucasian ancestry only, with seven well-spaced SNPs spanning the *PON* gene cluster (table 2). The probands of the trios also served as cases in the case-control analysis. In the trio analysis, the *PON3* SNP9 ($p = 0.014$) and the *PON2* SNP13 ($p = 0.031$) showed association with SALS. The LD structure comprised two blocks of high LD: one encompassing *PON1* (block I) and the other encompassing *PON3* and *PON2* (block II). These two blocks were separated by a region of low LD. In block II, there was a strong protective association of the haplotype AACT ($p = 7.14 \times 10^{-5}$) with SALS, whereas the haplotype AGCC was found to be detrimental ($p = 0.026$). No haplotype association was found in block I. In the case-control model, no association of any SNP was found with SALS. The haplotype CATT in block II was found to be associated with SALS ($p = 0.033$).

Fine-mapping. Having found an association signal, we genotyped additional SNPs in the *PON* gene cluster to localize the signal. An interesting biologic candidate gene 400 kbp upstream of the *PON* gene cluster on the negative strand is dynein, cytoplasmic, intermediate polypeptide 1 (*DNCI1*). We genotyped three HapMap-tagged SNPs in

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Table 2 *Association statistics of the genotyped polymorphisms in the trio and case-control models*

NCBI ref.	SNP no.	Alleles	HWE	χ^2 case-control	p	χ^2 trios	p
rs2237582†	1	A>G	0.76	0.086	0.7697	0.265	0.6069
rs662	2	C>T	0.36	0.498	0.4804	0.343	0.5583
rs854560	3	A>T	0.67	1.319	0.2508	1.573	0.2097
rs854565†	4	A>G	0.51	0.728	0.3936	0.444	0.5050
rs705382†	5	G, G	1.0	1.163	0.2808	2.063	0.1509
rs4141217	6	C>T	0.49	0.573	0.4489	0.143	0.7055
rs3757708†	7	G>T	0.42	1.588	0.2076	0.054	0.8164
rs978903	8	A>G	0.59	0.972	0.3243	0.277	0.5988
rs10487132†	9	A>G	1.0	2.015	0.1558	6.017*	0.0142*
rs2072200	10	C>G	0.47	0.655	0.4185	0.615	0.4328
rs6954345	11	C>G	0.99	2.676	0.1019	0.444	0.5050
rs2299263†	12	C>T	0.99	3.046	0.0810	1.043	0.3070
rs11981433†	13	C>T	0.52	0.09	0.7647	4.646*	0.0311*
rs2299267	14	A>G	0.21	0.62	0.4310	1.391	0.2383
rs1869050	15	A>T	0.49	1.994	0.1579	0.405	0.5245
rs1685818	16	G>T	0.08	1.066	0.3019	0.277	0.5988
rs2283010	17	A>G	0.98	3.261	0.0710	0.077	0.7815

* Significant p values.
† Single nucleotide polymorphisms genotyped for the preliminary screen.

NCBI = National Center for Biotechnology Information; SNP = single nucleotide polymorphism; HWE = Hardy-Weinberg equilibrium.

DNCI1 as well. Thus, a total of 17 SNPs were genotyped in the region on 744 Caucasian subjects (186 trios and 186 controls). The results verified the preliminary screen and showed that the signal was localized to *PON2-PON3*, a region in extensive LD (figure E-1 on the *Neurology* Web site at www.neurology.org).

In the trio group, apart from SNPs 9 and 13, no other SNP showed an association with SALS. These SNPs were in moderately high LD ($r^2 = 0.75$). Thus we considered their association to represent a single signal in the *PON* cluster with which both these SNPs were in LD. Because SNP9 had greater genotype and haplotype significance (tables 2 and 3) than SNP13, we removed SNP13 from the haplotype analysis to minimize redundancy.

By sliding window analysis, we found that SNPs 9, 10, 11, and 14 comprised a haploblock (encompassing the *PON2-PON3* region) that strongly associated with SALS. Two haplotypes in this block were found to be associated with SALS: GCCA was detrimental ($p = 0.017$), whereas ACCA was protective ($p = 0.001$). These two haplotypes differed only at SNP9, indicating that the G allele was detrimental and the A allele was protective.

In the case-control model, the haplotype ACAA comprising of SNPs 9, 10, 11, and 14 showed a minor association with SALS ($p = 0.037$), whereas none of the 17 SNPs showed genotype association. A trend toward an association was found with SNP11, a coding nonsynonymous SNP ($\chi^2 = 2.67$), which was confirmed by SNP12 ($\chi^2 = 3.05$) found to be in high LD with SNP11 ($r^2 = 0.99$). SNPs 10 and 14 showed no genotype association in the case-control or the trio model.

Replication of association. The association signal in the *PON* cluster was most strongly accounted for by SNPs 9 and 11. These SNPs, apart from comprising the associated haploblock, independently showed two-locus haplotype associations with several of the SNPs in the trio group (table 3). Although the haplotype signal was much reduced and had a different pattern in the case-control model compared to the trio group, SNP9 continued to show increased haplotype significance with SNPs 10 through 14. SNP12, which is in high LD with SNP11 also showed increased haplotype significance with several SNPs in *PON3* (table 3). Thus, we genotyped SNP9 and SNP11 on our entire cohort of Caucasian subjects (N = 1,891) to replicate the association signal.

Family based. Addition of 17 Caucasian trios (total n = 203) to the preliminary data set increased the significance for SNP9 (G allele, Z score = 2.843, $p = 0.0045$, FDR cutoff = 0.025). The DSP group (n = 247) did not show association (Z score = 1.223, $p = 0.22$, FDR cutoff = 0.025). When DSPs were combined with trios as 450 nuclear pedigrees, the association of the G allele was even stronger (Z score = 2.944, $p = 0.0032$, FDR cutoff = 0.0083), demonstrating that there was additional information in the DSPs that was not independently significant due to smaller sample size. SNP11 remained not significant in the trio ($p = 0.69$), DSP ($p = 0.76$), and pedigree models ($p = 0.62$). Haplotype analysis of SNP9 and SNP11 using HBAT showed significant associations (table 4).

Case-control. There was no association for either SNP in the independent case-control cohort of 284 cases and 504 controls ($P_{SNP9} = 0.72$, $P_{SNP11} = 0.52$). Haplotype analysis using THESIAS also did not demonstrate association ($\chi^2 = 2.42$, 3 df, $p = 0.12$). Including all probands (n = 734) and controls (n = 504) did not change the association statistics ($P_{SNP9} = 0.41$, $P_{SNP11} = 0.19$). Haplotype analysis showed lack of association as well ($\chi^2 = 2.97$, 3 df, $p = 0.40$). Subphenotype analysis (age at onset, site at onset)

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Table 3 *Two-locus haplotype p values for all genotyped polymorphisms in the trio and case-control*

	Case-controls																
1		0.55	0.27	0.23	0.21	0.43	0.29	0.35	0.23	0.39	0.16	0.16	0.32	0.22	0.14	0.16	0.07
2	0.52		0.25	0.16	0.14	0.37	0.20	0.27	0.20	0.27	0.26	0.12	0.16	0.18	0.13	0.14	0.05*
3	0.29	0.24		0.26	0.08	0.17	0.16	0.13	0.09	0.18	0.11	0.11	0.34	0.13	0.08	0.19	0.06
4	0.30	0.17	0.42		0.29	0.35	0.21	0.25	0.12	0.30	0.08	0.08	0.21	0.35	0.16	0.25	0.11
5	0.20	0.09	0.09	0.09		0.27	0.19	0.25	0.19	0.14	0.10	0.11	0.12	0.16	0.12	0.11	0.07
6	0.05*	0.12	0.14	0.16	0.07		0.15	0.37	0.26	0.12	0.11	0.05*	0.13	0.16	0.13	0.23	0.10
7	0.03*	0.09	0.10	0.18	0.01*	0.07		0.18	0.17	0.05*	0.06	0.04*	0.09	0.06	0.06	0.15	0.05*
8	0.03*	0.10	0.08	0.16	0.05*	0.53	0.07		0.20	0.09	0.08	0.05*	0.15	0.12	0.10	0.18	0.09
9	0.04*	0.03*	0.005*	0.04*	0.02*	0.006*	0.0005*	0.004*		0.04*	0.06	0.04*	0.02*	0.05*	0.09	0.11	0.05*
10	0.29	0.31	0.19	0.12	0.09	0.46	0.60	0.47	0.005*		0.10	0.10	0.32	0.25	0.11	0.26	0.10
11	0.06	0.03*	0.08	0.27	0.02*	0.25	0.69	0.25	0.005*	0.55		0.09	0.13	0.09	0.05*	0.09	0.02*
12	0.05*	0.02*	0.08	0.14	0.008*	0.08	0.46	0.06	0.0005*	0.31	0.33		0.09	0.08	0.06	0.08	0.02*
13	0.05*	0.06	0.04*	0.06	0.05*	0.003*	0.0004*	0.003*	0.01*	0.01*	0.003*	0.0006*		0.35	0.26	0.32	0.19
14	0.10	0.14	0.14	0.18	0.05*	0.31	0.51	0.37	0.01*	0.27	0.36	0.20	0.02*		0.16	0.28	0.07
15	0.34	0.40	0.05*	0.19	0.08	0.34	0.44	0.29	0.02*	0.09	0.15	0.09	0.07	0.11		0.06	0.04*
16	0.40	0.40	0.10	0.34	0.33	0.23	0.29	0.21	0.001*	0.16	0.28	0.26	0.01*	0.19	0.58		0.09
17	0.21	0.18	0.12	0.16	0.19	0.17	0.21	0.11	0.01*	0.27	0.38	0.29	0.03*	0.23	0.07	0.12	
SNP	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Trios																

* Significant *p* values.

All single nucleotide polymorphism pair combinations were tested for association using haplotype family based association test (HBAT) for the trio group and Haploview for the case-control group. The lowest *p* values for the haplotypes with a frequency >0.01 are tabulated. Retesting of the trio group two-locus statistics with Haploview showed high concordance with HBAT.

also showed a lack of significance for both SNPs across all models employed (table 5).

Resequencing. Using HelixTree, 25 SALS patients who carried the detrimental haplotype GCCA and 16 patients who carried the protective haplotype ACCA were identified. Of these, 10 patients were homozygous for GCCA, whereas only two were homozygous for ACCA. We resequenced all exons of *PON3* and *PON2* genes in these 12 patients to identify potentially functional variants. We could not identify any new sequence change in *PON2* or *PON3*. None of the coding variants differed between these two groups of patients. Apart from SNP9, an intronic SNP in *PON2* rs17876088 did differ between the two groups. All 10 patients homozygous for GCCA were also homozygous for the C allele of rs17876088, whereas the patients homozygous for ACCA were homozygous for the A allele of this polymorphism.

Discussion. This study demonstrates significant evidence of association of variants in the *PON* gene cluster with SALS. Our results identify a specific genomic region and haplotypes within the *PON* locus that may harbor biologically significant variant(s). This region comprises the haploblock of high LD spanning *PON2* and *PON3*. The G allele of SNP9 (rs10487132) was consistently associated with SALS in the family-based models. Inconsistency in replication is a well-recognized phenomenon in case-control genetic association studies because testing allelic as-

Table 4 *HBAT haplotype (SNP9 and SNP11) statistics for SALS trios, discordant sibpairs and pedigrees*

Haplotype	Frequency	FDR cutoff	Trios *Z* (*p*) F = 203; n = 609	DSP *Z* (*p*) F = 247; n = 494	Pedigrees *Z* (*p*) F = 450; n = 1,133
H1	0.371	0.0167	−3.0 (0.0027)*	−1.50 (0.15)	−3.26 (0.0011)*
H2	0.354	0.0333	2.74 (0.0062)*	1.24 (0.22)	2.85 (0.0043)*
H3	0.274	0.0500	0.5 (0.61)	0.28 (0.78)	0.58 (0.56)

Numbers in parentheses indicate *p* values.

* Significant.

FDR cutoff = false discovery rate *p* value cutoff for multiple testing; F = the number of nuclear families included in the analysis; n = the number of subjects analyzed; f = haplotype frequency; *Z* = haplotype family based association test (HBAT) *Z* statistic.

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Table 5 *Association of SALS subphenotypes with PON SNPs 9 and 11*

SALS subphenotype	rs10487132 SNP9 χ^2 (p)	rs6954345 SNP11 χ^2 (p)
Bulbar/spinal onset	3.43 (0.18)	5.71 (0.06)
UL/LL onset	1.87 (0.39)	1.14 (0.57)
EOALS/LOALS	3.93 (0.14)	1.72 (0.42)
Age at onset*	0.31 (0.58)	1.22 (0.27)

* Age at onset was analyzed as a quantitative trait by Kaplan-Meier procedure. Log-rank test with 1 *df* is reported.

SALS = sporadic ALS; UL = upper limb; LL = lower limb; EOALS = early-onset ALS; LOALS = late-onset ALS.

sociation with disease using unrelated case-control samples is prone to selection bias and population stratification.[29] A family-based design protects against such spurious associations. The TDT overcomes the problem of an adequate match between the controls and the cases by using family members as comparison groups. Moreover, TDT tests for linkage and association simultaneously and a positive result indicates that a susceptibility allele is nearby. We could replicate the association with SNP9 by addition of trios and discordant sibpairs. Our case-control models, however, remained negative despite our large sample size. This may be due to hidden population substructure, significant age difference between the two groups (table 1), or any other unmatched variable(s) resulting in a different LD structure than in the trio model (table 3). On resequencing, another intronic polymorphism in *PON2* was identified that could differentiate between the protective and the detrimental haplotypes; however, no coding variation could account for the association. This pattern of association appears to be complex and a combination of several variants (haplotype) across the *PON* cluster may be needed to confer susceptibility to ALS.

PON cluster variants have previously been associated with other neurodegenerative and vascular disorders including Alzheimer disease (AD),[30] Parkinson disease,[31] coronary artery disease,[32] and stroke.[33,34] The missense coding polymorphisms Q192R (SNP2), L55M (SNP3) in *PON1* and C311S (SNP11) in *PON2* have been most often implicated in these disorders; however, we did not find these to be associated with ALS and neither did a recent study on AD.[30] However, as part of haplotypes with SNP9, these SNPs may confer susceptibility to ALS, as shown for SNP11 and by two-locus haplotype analysis (table 3).

Although the mechanism by which *PON* genes may increase susceptibility to ALS is not clear, several studies have previously implicated *PON1* polymorphisms, serum levels, and activity in the neurologic manifestations of organophosphate poisoning.[15-20] These studies, unlike ours, did not comprehensively explore the genetic variation in the *PON* cluster but were rather limited to a few coding variants in one or two genes. There is extensive LD in the *PON* cluster region, allowing the association signal to be picked up by different variants depending on the population under study, the genetic model employed, and the statistical methodologies. Moreover, ALS risk may in fact be modulated by a *PON* cluster haplotype rather than a single variant in a single gene. Recently, reports describe a possible twofold increased risk of developing ALS in Gulf War veterans.[11,12] These studies had drawbacks in design,[13,14] but hint toward a war related environmental exposure (organophosphate pesticides and chemical nerve agents such as sarin) in a genetically susceptible host,[10] as a possible etiologic factor. Although our sample was not analyzed for inclusion of Gulf War veterans, our finding of a robust association in the *PON* gene cluster is consistent with the hypothesis of environmental toxicity in a susceptible host precipitating ALS.

Enzymatic characterization of the purified PONs has revealed them to be lactonases, with overlapping substrates (e.g., aromatic lactones) and also distinctive substrate specificities.[2] The *PON* gene family shows high similarity in their structural characteristics and has 65% identity at the amino acid level,[35] and 81 to 95% nucleotide conservation across mammalian species.[35-37] These findings suggest an important concerted physiologic role(s) for PONs.

PON1 hydrolyzes the toxic oxon metabolites of a number of organophosphate insecticides such as parathion, diazinon, and chlorpyrifos and even nerve gas agents such as sarin and soman.[38] Crystallization studies showed that amino acid variation in various parts of the PON1 structure markedly shifts its substrate specificity and reactivity.[39] Thus, certain substrates are metabolized rapidly, whereas others more slowly. A well-known example is the Q192R (SNP2) polymorphism where the Q form is more efficient at hydrolyzing diazoxon, sarin, and soman, whereas the R form more efficiently hydrolyzes paraoxon produced from the insecticide parathion.[38]

The complex pattern of association revealed in our study may be due to the intricate variability of substrate specificity of PONs. Different classes of environmental exposures (e.g., sarin and paraoxon) may be having the same effect, albeit through different genetic polymorphisms as is known for the Q192R variant.[2,38] In such a case, consistent association with a single variant may be difficult to replicate as in our case-control model. Multiple splice variants as in *PON2* may enhance this complexity.[40] It has been shown that measuring enzyme activity and serum levels has additional value in delineating this complexity and may provide further insights into the role of PONs in ALS.[41,42] Thus, SNP genotyping in the intergenic regions of the *PON* gene cluster and its replication, gene expression, gene-gene interaction, and PON serum/enzymatic studies may help elucidate the complexity of *PON* cluster association with ALS.

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.

Acknowledgment

The authors thank the patients with ALS and their families for their participation.

References

1. Siddique T, Pericak-Vance MA, Brooks BR, et al. Linkage analysis in familial amyotrophic lateral sclerosis. Neurology 1989;39:919–925.
2. Draganov DI, Teiber JF, Speelman A, Osawa Y, Sunahara R, La Du BN. Human paraoxonases (PON1, PON2, and PON3) are lactonases with overlapping and distinct substrate specificities. J Lipid Res 2005; 46:1239–1247.
3. Costa LG, Cole TB, Furlong CE. Polymorphisms of paraoxonase (PON1) and their significance in clinical toxicology of organophosphates. J Toxicol Clin Toxicol 2003;41:37–45.
4. Clendenning JB, Humbert R, Green ED, Wood C, Traver D, Furlong CE. Structural organization of the human PON1 gene. Genomics 1996; 35:586–589.
5. Reddy ST, Wadleigh DJ, Grijalva V, et al. Human paraoxonase-3 is an HDL-associated enzyme with biological activity similar to paraoxonase-1 protein but is not regulated by oxidized lipids. Arterioscler Thromb Vasc Biol 2001;21:542–547.
6. Ng CJ, Wadleigh DJ, Gangopadhyay A, et al. 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.
7. Costa LG, Li WF, Richter RJ, Shih DM, Lusis A, Furlong CE. The role of paraoxonase (PON1) in the detoxication of organophosphates and its human polymorphism. Chem Biol Interact 1999;119-120:429–438.
8. Furlong CE, Richter RJ, Seidel SL, Motulsky AG. Role of genetic polymorphism of human plasma paraoxonase/arylesterase in hydrolysis of the insecticide metabolites chlorpyrifos oxon and paraoxon. Am J Hum Genet 1988;43:230–238.
9. Humbert R, Adler DA, Disteche CM, Hassett C, Omiecinski CJ, Furlong CE. The molecular basis of the human serum paraoxonase activity polymorphism. Nat Genet 1993;3:73–76.
10. Li WF, Costa LG, Richter RJ, Hagen T, et al. Catalytic efficiency determines the in-vivo efficacy of PON1 for detoxifying organophosphorus compounds. Pharmacogenetics 2000;10:767–779.
11. Haley RW. Excess incidence of ALS in young Gulf War veterans. Neurology 2003;61:750–756.
12. Horner RD, Kamins KG, Feussner JR, et al. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. Neurology 2003;61:742–749. Erratum in Neurology 2003;61:1320.
13. Rose MR. Gulf War service is an uncertain trigger for ALS. Neurology 2003;61:730–731.
14. Armon C. Excess incidence of ALS in young Gulf War veterans. Neurology 2004;63:1986–1987; author reply 1986–1987.
15. Haley RW, Billecke S, La Du BN. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. Toxicol Appl Pharmacol 1999;157:227–233.
16. Furlong CE. PON1 status and neurologic symptom complexes in Gulf War veterans. Genome Res 2000;10:153–155.
17. Haley RW. Gulf war syndrome: narrowing the possibilities. Lancet Neurol 2003;2:272–273.
18. Hotopf M, Mackness MI, Nikolaou V, et al. Paraoxonase in Persian Gulf War veterans. J Occup Environ Med 2003;45:668–675.
19. Mackness B, Durrington PN, Mackness MI. Low paraoxonase in Persian Gulf War Veterans self-reporting Gulf War syndrome. Biochem Biophys Res Commun 2000;276:729–733.
20. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the molecular polymorphisms of human paraoxonase (PON1) on the rate of hydrolysis of paraoxon. Br J Pharmacol 1997;122:265–268.
21. Brooks BR. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical Limits of Amyotrophic Lateral Sclerosis" workshop contributors. J Neurol Sci 1994;124 Suppl:96–107.
22. Sambrook J, Fritsch EF, Maniatis T. Molecular cloning, a laboratory manual. 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1989.
23. Storey JD, Tibshirani R. Statistical significance for genomewide studies. Proc Natl Acad Sci USA 2003;100:9440–9445.
24. Laird NM, Horvath S, Xu X. Implementing a unified approach to family-based tests of association. Genet Epidemiol 2000;19 (suppl 1): S36–42. Abstract.
25. Tregouet DA, Escolano S, Tiret L, Mallet A, Golmard JL. A new algorithm for haplotype-based association analysis: the stochastic-EM algorithm. Ann Hum Genet 2004;68:165–177.
26. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–265.
27. HelixTree version 4.2.2 GoldenHelix Inc. [online] Available at: http://www.goldenhelix.com/pharmhelixtreefeatures.html. Accessed January 19, 2006.
28. Li YJ, Pericak-Vance MA, Haines JL, et al. Apolipoprotein E is associated with age at onset of amyotrophic lateral sclerosis. Neurogenetics 2004;5:209–213.
29. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nat Genet 2001;29: 306–309.
30. Erlich PM, Lunetta KL, Cupples LA, et al. Polymorphisms in the PON gene cluster are associated with Alzheimer disease. Hum Mol Genet 2006;15:77–85.
31. Zintzaras E, Hadjigeorgiou GM. Association of paraoxonase 1 gene polymorphisms with risk of Parkinson's disease: a meta-analysis. J Hum Genet 2004;49:474–481.
32. Wheeler JG, Keavney BD, Watkins H, Collins R, Danesh J. Four paraoxonase gene polymorphisms in 11212 cases of coronary heart disease and 12786 controls: meta-analysis of 43 studies. Lancet 2004;363:689–695.
33. Ranade K, Kirchgessner TG, Iakoubova OA, et al. Evaluation of the paraoxonases as candidate genes for stroke: Gln192Arg polymorphism in the paraoxonase 1 gene is associated with increased risk of stroke. Stroke 2005;36:2346–2350.
34. Voetsch B, Benke KS, Damasceno BP, Siqueira LH, Loscalzo J. Paraoxonase 192 Gln–>Arg polymorphism: an independent risk factor for nonfatal arterial ischemic stroke among young adults. Stroke 2002;33: 1459–1464.
35. Primo-Parmo 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.
36. La Du BN, Aviram M, Billecke S, et al. On the physiological role(s) of the paraoxonases. Chem Biol Interact 1999;119-120:379–388.
37. Draganov DI, La Du BN. Pharmacogenetics of paraoxonases: a brief review. Naunyn Schmiedebergs Arch Pharmacol 2004;369:78–88.
38. Davies HG, Richter RJ, Keifer M, Broomfield CA, Sowalla J, Furlong CE. The effect of the human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. Nat Genet 1996;14:334–336.
39. Harel M, Aharoni A, Gaidukov L, et al. Structure and evolution of the serum paraoxonase family of detoxifying and anti-atherosclerotic enzymes. Nat Struct Mol Biol 2004;11:412–419.
40. Mochizuki H, Scherer SW, Xi T, et al. Human PON2 gene at 7q21.3: cloning, multiple mRNA forms, and missense polymorphisms in the coding sequence. Gene 1998;213:149–157.
41. Jarvik GP, Jampsa R, Richter RJ, et al. Novel paraoxonase (PON1) nonsense and missense mutations predicted by functional genomic assay of PON1 status. Pharmacogenetics 2003;13:291–295.
42. Jarvik GP, Rozek LS, Brophy VH, et al. Paraoxonase (PON1) phenotype is a better predictor of vascular disease than is PON1(192) or PON1(55) genotype. Arterioscler Thromb Vasc Biol 2000;20:2441–2447.

Copyright © by AAN Enterprises, Inc. Unauthorized reproduction of this article is prohibited.