Supplementary Material

1. Full Introduction

Paraoxonase 1 (PON1) has been implicated in a variety of neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis. The enzyme was originally identified due to its ability to hydrolyze paraoxon, an organophosphate pesticide. PON1 is synthesized by the liver and is abundant in serum, where it inhibits oxidation of low-density lipoprotein and associates with HDL (Durrington, et al., 2001,Mackness, et al., 2001). PON1 activity varies 10- to 40-fold between individuals, a result of several single nucleotide polymorphisms (SNPs). The PON1 Q192R polymorphism (rs662) has been associated with differential metabolism of organophosphates. For instance, RR192 homozygous individuals metabolize paraoxon rapidly and diazoxon slowly compared with those who are QQ192 homozygous, while the rates of metabolism of other organophosphates are similar between the two isozymes (Davies, et al., 1996). The L55M polymorphism (rs854560) is associated with increased levels of PON1 expression in the serum, possibly due to linkage with the C107T (rs705379) polymorphism in the promoter, which itself explains 22.8% of the variance in serum expression (Brophy, et al., 2001). Nevertheless, the exact physiologic role of PON1 and its coding polymorphisms is still not entirely clear. Mice that lack PON1 show evidence of greater oxidative stress and organophosphate toxicity (Shih, et al., 1998). In humans, a recent prospective study of patients undergoing coronary angiography showed decreased levels of PON1 activity and increased levels of oxidative stress in individuals homozygous for QQ192 compared with QR192 and RR192 genotypes (Bhattacharyya, et al., 2008), making a more compelling case for PON1 in the development of atherosclerosis.

Given the putative role of PON1 in oxidative stress and atherosclerosis, as well as epidemiologic and pathologic studies linking Alzheimer's disease and atherosclerosis to PON1 (Esiri, et al., 1999,Hofman, et al., 1997), 13 studies have explored the association between

PON1 and AD. Of those, five case-control studies and one family-based study reported an association between PON1 polymorphisms and AD (Chapuis, et al., 2009,Erlich, et al., 2006,He, et al., 2006,Leduc and Poirier, 2008,Leduc, et al., 2009,Scacchi, et al., 2003), while seven case-control studies showed no association, making the relationship between PON1 and AD uncertain (Cellini, et al., 2006,Dantoine, et al., 2002,Helbecque, et al., 2004,Pola, et al., 2003,Sodeyama, et al., 1999,Yamada, 2002,Zuliani, et al., 2001). Ten of these studies genotyped one or both coding SNP in PON1 rs854560 (L55M) and rs662 (Q192R). One genome-wide association study found a trend for association for another PON1 SNP (Li, et al., 2008) that did not survive correction for multiple tests.

The ability of PON1 to metabolize pesticides has also prompted investigations into possible links with Parkinson's disease, since pesticide exposure is a risk factor for development of the disease. Similar to AD, finding an association between PON1 polymorphisms and PD has met with mixed results. A recent meta-analysis found the rs854560 55M allele conferred an increased risk of developing PD compared with the 55L allele, with an odds ratio of 1.32 (1.10-1.59, 95%CI), but found no association with rs662 (Q192R) and no evidence of publication bias (Zintzaras and Hadjigeorgiou, 2004). Interestingly, a recent case-control study examining environmental exposures, including pesticides and PON1 polymorphisms in PD, suggested an association between the rs854560 polymorphism and pesticide exposure based on very small numbers of exposed subjects (Dick, et al., 2007).

Previous case-control association studies of PON1 in AD and PD were limited to detecting relatively large effect sizes, due to their small sample size or the need to correct for multiple tests. Hence we examined two PON1-coding SNPs in our combined cohort of AD and Caucasian cohort of PD patients with 80% power to detect a relative risk of 1.25 and 1.35, respectively (Purcell, et al., 2003). Because these diseases share a variety of epidemiological,

clinical, and pathological features, we hypothesized that the PON1 variants previously associated with AD or PD could confer additional risk for the spectrum of cases that share characteristics of both AD and PD. We define these AD-PD overlap diseases as clinically diagnosed cases of dementia with Lewy bodies (DLB), Parkinson's disease with dementia (PDD), and Alzheimer's disease with Parkinson's disease (ADPD). To test our hypothesis we ascertained individuals with AD, PD, AD-PD overlap disorder, and healthy age-matched controls, and genotyped them for rs662 (R192Q) and rs854560 (L55M). We used a logistic regression model adjusting for relevant covariates and studied the association between PON1 polymorphism and AD, PD, or AD-PD overlap disorders. Since we had already shown that individuals with a family history of either AD or PD in this cohort have higher odds of developing either disease (Rosen, et al., 2007), we also assessed the possibility that PON1 contributes to this shared familial risk.

2. Full Methods

Subjects

From 1992 to 2008, participants with Alzheimer's and Parkinson's diseases were recruited from the Emory University Department of Neurology specialty clinics, including the Memory Disorders Clinic, Movement Disorders Clinic, and Alzheimer's Disease Research Center (ADRC). All individuals who presented to one of the clinics or ADRC were eligible for enrollment and underwent an evaluation including a history and neurologic examination including a motor Unified Parkinson's Disease Rating Scale. All individuals underwent initial cognitive screening with a Mini-Mental State Exam (MMSE) and Clock Drawing Test (CDT), and those who were impaired ($z \leq -1.79$) adjusted for age, sex, and education underwent further neuropsychological testing consisting of a Brief Visuospatial Memory Test Revised, Wechsler Memory Scale Revised Logical Memory I and II, Wechsler Adult Intelligence Scale Revised Similarities, Wechsler Adult Intelligence Scale III Digit Span, Wechsler Adult Intelligence Scale Revised Digit

Symbol, Judgment of Line Orientation, Trail Making Test A and B, Category Fluency (Animals, Vegetables), Phonemic Fluency (FAS), Boston Naming Test, CERAD Word List Memory, CDT, Beck Depression Inventory (for individuals <65 years), and Geriatric Depression Scale (for individuals ≥65 years). All individuals presenting with parkinsonism or cognitive complaints underwent neuroimaging with an MRI or CT along with routine laboratory blood work to exclude other causes of their symptoms. All controls reported no personal history of any neurologic disease, and medical records that included neuroimaging (CT or MRI), neuropsychiatric assessment, and ancillary testing were requested and reviewed when available. The exclusion criterion was that only one individual per immediate family was eligible for enrollment.

Diagnosis

A research diagnosis of AD, PD, AD-PD, DLB, PDD, or control was assigned by an experienced neurologist with subspecialty training in cognitive or movement disorders who examined the patient, and confirmed by another subspecialty trained neurologist after reviewing all available medical records. Any discrepant diagnosis was resolved by consensus. The research diagnosis of dementia was based upon the Diagnostic and Statistical Manual of Mental Disorders 4th edition (DSM-IV), and AD, PD, and DLB were determined based on established criteria (Gelb, et al., 1999,McKeith, 2006,Morris, et al., 1989). Parkinson's disease with dementia was defined as the development of dementia based on DSM-IV criteria at least one year after the onset of motor symptoms. The diagnosis of AD with PD was based on the development of motor symptoms of PD at least one year after the onset of cognitive symptoms. Controls were defined as individuals without any memory or movement complaints confirmed by an informant or medical records and normal performance on the MMSE (≥ 27) and a CDT (≥ 11).

Genotyping

TaqMan assays for two SNPs within PON1, rs662 and rs854560, were purchased from Applied Biosystems (Foster City, CA). All enrolled individuals were assayed on an ABI 7700 system using the manufacturer's standard protocol. Genotyping was performed twice on 1139 individuals for each assay to determine the concordance. APOE genotyping was performed by a standard protocol (Hixson and Vernier, 1990).

Statistical Analysis

All analyses were performed using PLINK (Purcell,Purcell, et al., 2007). Since we collected an ethnically mixed group of individuals we choose to limit our analysis to cases where we recruited >100 controls or >25 cases as the cut-off for subsequent analysis to avoid false-positive results due to population substructure or inability to control for covariates. In African Americans and Caucasians each SNP was tested for Hardy-Weinberg equilibrium. First, allelic associations between each SNP and disease were tested by χ^2 analysis. Next, logistic regression was performed on Caucasians for each disease outcome and African Americans for AD. Additive and genotypic models were tested adjusting for age, sex, ethnicity, number of APOE ε4 alleles, and years of education. For AD, a fixed-effects and random-effects meta-analysis was performed on both SNPs as implemented by PLINK using the results of the logistic regression models. Lastly, we tested for association between PON1 genotype and individuals with a family history of AD and PD for each disease category using Fisher's exact tests.

Ethics

All participants provided written informed consent, except those who lacked decision-making capacity. The latter group provided verbal assent, and written informed consent was obtained from a legal representative. This study was approved by the Emory University Institutional Review Board prior to the study start date.

Supplemental Table 1: Study Demographics

Characteristic		Control (n=719)	AD (n=746)	PD (n=566)	AD-PD Overlap (n=135)
Age mean, (SD), y		73.6 (10.2)	80.3 (9.0)	69.9 (11.2)	77.5 (8.1)
Sex, No. (%)	Male	240 (33.4%)	267 (35.8%)	359 (63.4%)	84 (63.6%)
	Female	479 (66.6%)	479 (64.2%)	207 (36.6%)	48 (36.4%)
Ethnicity, No. (%)	African American	135 (18.8%)	208 (27.9%)	0 (0%)	0 (0%)
	Caucasian	584 (81.2%)	538 (72.1%)	566 (100%)	135 (100%)
Education, Median (Interquartile Range), y[1]		16 (5)	12 (4)	16 (4)	14 (4)
No. of APOE ε4 alleles, No. (%)[2]	0	415 (57.7%)	242 (32.4%)	405 (65.1%)	71 (53.8%)
	1	181 (25.2%)	357 (32.44%)	120 (21.2%)	44 (33.3%)
	2	15 (2.1%)	98 (13.14%)	10 (1.8%)	2 (1.52%)

[1] 11.7% overall missing data for Education

[2] 10.7% overall missing data for APOE4 allele

Supplemental Table 2: Logistic Regression Results for rs662

		Alzheimer's Disease		Parkinson's Disease		ADPD	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Caucasian	Genotypic	N/A	0.8738	N/A	0.5112	N/A	0.5703
	Additive (allele C)	0.9481 (0.7278-1.235)	0.6928	0.958 (0.75-1.224)	0.7311	0.7881 (0.5043-1.232)	0.2958
	Age	1.052 (1.035-1.069)	5.183e-10	0.9456 (0.9324-0.959)	7.146e-15	1.03 (1.004-1.056)	0.02163
	Sex – male vs. female	0.7416 (0.5481-1.004)	0.05278	0.3006 (0.2273-0.3975)	3.443e-17	0.2861 (0.1841-0.4446)	2.653e-08
	No. of ApoE4 Alleles	3.419 (2.682-4.36)	3.568e-23	0.7269 (0.553-0.9557)	0.02232	1.395 (0.9393-2.072)	0.099
	Years of Formal Education	0.8343 (0.7927-0.878)	3.683e-12	0.8974 (0.8562-0.9405)	6.284e-06	0.8356 (0.7748-0.9012)	3.168e-06
African American	Genotypic	N/A	0.7774				
	Additive (allele T)	0.8195 (0.4568-1.47)	0.5044				
	Age	1.172 (1.125-1.221)	3.068e-14				
	Sex – male vs. female	1.147 (0.544-2.417)	0.719				
	No. ApoE4 Alleles	3.739 (2.167-6.452)	2.162e-06				
	Years of Formal Education	0.8596 (0.7879-0.9379)	0.0006704				

ADPD = individuals with a diagnosis of AD-PD, DLB, or PDD; OR = odds ratio; CI = confidence interval

Supplemental Table 3: Logistic Regression Results for rs854560

		Alzheimer's Disease		Parkinson's Disease		ADPD	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Caucasian	Genotypic	N/A	0.5351	N/A	0.7192	N/A	0.4488
	Additive (allele T)	0.8813 (0.6985-1.112)	0.2869	0.9675 (0.7848-1.193)	0.7573	1.234 (0.8896-1.712)	0.2077
	Age	1.053 (1.036-1.07)	2.629e-10	0.9471 (0.9338-0.9605)	3.647e-14	1.029 (1.003-1.056)	0.02717
	Sex – male vs. female	0.7293 (0.5383-0.9881)	0.04162	0.2904 (0.2195-0.3842)	4.707e-18	0.2745 (0.1754-0.4294)	1.499e-08
	No. ApoE4 Alleles	3.438 (2.694-4.386)	3.008e-23	0.7279 (0.554-0.9563)	0.02257	1.398 (0.9381-2.082)	0.09981
	Years of Formal Education	0.8318 (0.7902-0.8757)	2.202e-12	0.894 (0.8529-0.937)	3.007e-06	0.830 (0.769-0.8958)	1.707e-06
African American	Genotypic	N/A	0.9759				
	Additive (allele T)	0.9356 (0.4105-2.132)	0.8742				
	Age	1.168 (1.121-1.216)	7.144e-14				
	Sex – male vs. female	1.097 (0.5269-2.285)	0.8043				
	No. ApoE4 Alleles	3.569 (2.083-6.114)	3.613e-06				
	Years of Formal Education	0.8521 (0.7813-0.9294)	0.0003015				

ADPD = individuals with a diagnosis of AD-PD, DLB, or PDD; OR = odds ratio; CI = confidence interval

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