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Glutathione S-transferase mu, omega, pi, and theta class variants and smoking in Parkinson's disease.

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Abstract

GSTs are a family of inducible phase II enzymes that may play a neuroprotective role in Parkinson's disease (PD). GSTs may also modify PD risk by metabolizing compounds in cigarettes, as cigarette smoking is generally found to be associated with a decrease in PD risk. Using a population-based case control study design, we examined polymorphisms of the mu, omega, pi, and theta classes of GST to elucidate the main effects and smoking-GST interactions on PD risk. From three rural California counties, we recruited 289 incident idiopathic PD cases, clinically confirmed by our study neurologist, and 270 population controls, marginally matched by age, gender, and race.

We assessed main gene polymorphism associations and evaluated interactions between smoking and GST polymorphisms as departures from a multiplicative scale adjusting for age, gender, and race. We also restricted analyses to Caucasian subjects to address the potential for population stratification (n=235 cases, 220 controls).

Among Caucasians, we observed a risk reduction in subjects carrying at least one variant allele for GSTO1 (OR= 0.68, 95% CI: 0.47–0.98) and also GSTO2 (OR= 0.64, 95% CI: 0.44–0.93); both genes were in strong linkage disequilibrium. No main gene effects were observed for the remaining polymorphisms. We noted a multiplicative interaction between ever having smoked regularly and GSTO1 ($OR_{interaction}$= 0.55, 95% CI: 0.33–0.92) and GSTO2 ($OR_{interaction}$= 0.54, 95% CI: 0.32–0.90). Results were similar when combining all races. These findings and the paucity of similar studies suggests a need for further inquiry into the association between GSTs, smoking, and PD risk.

Keywords

Gene-environment interaction; Glutathione-S-transferase; Parkinson's disease; Smoking

The death of dopaminergic neurons in the midbrain is a characteristic feature of Parkinson's disease (PD). Glutathione-S-transferases (GSTs), a family of inducible phase II enzymes with cytoprotective properties, are hypothesized to protect against neurodegeneration. GSTs may

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prevent dopaminergic degeneration through their direct antioxidant activity against various reactive metabolites of chemical toxicants produced by phase I enzyme metabolism [8,23, 17]. GSTs may also serve a neuroprotective function by facilitating the elimination of endogenous toxins, including the toxic *o*-quinones of dopamine, from the cell [1].

Furthermore, GSTs may modify PD risk by metabolizing some chemical compounds in tobacco smoke, since cigarette smokers generally have a lower risk of developing PD [22]. GST polymorphisms may account for different abilities to metabolize components of cigarette smoke, and thus alter an individual's susceptibility to PD. GSTs are known to catalyze various active metabolites of tobacco smoke such as benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons, and monohalomethanes [26]. The small number of studies that previously explored interactions between GST gene polymorphisms and smoking in PD were inconclusive and subject to limitations in study design and sample size [3,4,13]. Here we present the first population-based case control study to examine the interaction between several GST polymorphisms and smoking in PD.

We concentrated on polymorphisms in four of eight classes of cytoplasmic mammalian GSTs (mu, omega, pi, theta). These classes and their respective gene polymorphisms were selected according to the candidate gene approach, whereby we considered the gene polymorphism's biological relevance, allelic frequency, and implication in previous PD epidemiologic studies. Specifically, we evaluated the homozygous deletions GSTM1*0 and GSTT1*0 which result in a lack of enzyme activity [20,25]. Additionally we examined two single nucleotide polymorphisms in GSTP1 resulting in amino acid changes that may have an impact on gene function (rs947894, which results in an $Ile^{104}Val$ amino acid change; and rs1799811, which results in an $Ala^{114}Val$ amino acid change). Finally we examined a non-synonymous polymorphism of the GSTO1 gene (rs4925, which results in an $Ala^{140}Asp$ amino acid change), and a transition polymorphism in the GSTO2 gene (rs2297235, -183A to G) [29].

We also evaluated smoking-GST polymorphism interactions that involved a departure from multiplicativity. Each of the GST classes selected has previously been shown to interact with cigarette smoking in the context of cancer [11,12,26,31], and metabolic substrates for each GST class have been proposed or identified among the myriad components of cigarette smoke [11,28,7]. To the best of our knowledge, this is the first study to examine the interaction of the GST omega class and smoking in PD.

We recruited 289 incident idiopathic PD cases (enrolled within 3 years of diagnosis) and 270 controls between January 2001 and April 2006 as part of UCLA's Parkinson's Environment and Gene (PEG) Study, a population-based case-control study based in three rural California counties (Fresno, Tulare, and Kern). Age and gender matched controls were randomly selected from either Medicare records or residential parcels sampled from shape files of the tri-county area. The population accrued was primarily Caucasian (81.4%). The median age of cases at diagnosis was 70.1 years. The Caucasian population consisted of 235 incident cases (mean age 69.5 years, 54.0% male) and 220 controls (mean age 67.1 years, 52.5% male). See Table 1 for study population characteristics.

A diagnosis of clinically probable or possible PD was confirmed by a UCLA movement disorder specialist if patients met the following criteria: (1) manifestation of at least two of the following characteristics: resting tremor, bradykinesia, or cogwheel rigidity, at least one of which was resting tremor or bradykinesia; (2) no suggestion of a parkinsonian syndrome due to trauma, brain tumor, infection, cerebrovascular disease, or other known neurological disease, and no treatment in the past with dopamine-blocking or dopamine-depleting agents; (3) no atypical features such as prominent oculomotor palsy, cerebellar signs, vocal cord paresis, severe orthostatic hypotension, pyramidal signs, amyotrophy or limb apraxia; (4) asymmetric

onset; and (5) if treatment with levodopa had been initiated, symptomatic improvement after treatment. Probable cases met criteria (1) through (4) plus/minus (5). Possible cases had at least one sign from criterion (1) and fulfilled criteria described in (2) and (3). Although sometimes included under criterion (1), postural reflex impairment was excluded as a criterion because it usually occurs late in PD and may typically occur early in other parkinsonian disorders (i.e. Multiple System Atrophy and vascular Parkinsonism) [10].

Genetic analysis was performed on blood or buccal samples provided by all participants. Basic demographic and risk factor data were collected through telephone interviews by interviewers blinded to case/control status. Subjects who reported to have smoked cigarettes for more than one year were considered ever-regular smokers. All subjects provided written informed consent, and the UCLA ethics committee approved the study.

Blood/buccal samples were stored and processed at the UCLA Biologic Specimen Core Facility. All GST polymorphisms were genotyped via pyrosequencing using established methods [2,16,30]. SNPs and their NCBI SNP reference numbers are noted in Table 1, and further information including primer sequences and fragment lengths are available upon request.

Hardy-Weinberg equilibrium was assessed in cases and controls using the chi-squared test. Departure from Hardy-Weinberg equilibrium may be indicative of genotyping error or non-random selection of controls in terms of the distribution of a given polymorphism. We used logistic regression to assess main gene effects for each genotype. We also assumed a dominant inheritance model for each polymorphic site, assessing PD risk among those carrying one or more variant alleles compared to the wildtype genotype. All effect estimates were adjusted for age, gender, and race. Using stratified analysis, we evaluated subgroups of gender, age at diagnosis (≤60 yrs, >60 yrs), ever-regular smoking, and pack-years of smoking. We also restricted some analyses to those individuals with no family history of PD, which was defined as no first degree relative with PD. In order to evaluate interactions between smoking and genotypes on a multiplicative scale, we introduced indicator variables for each smoking/genotype level and an interaction term into models. Fischer's Exact Test was used to test for linkage disequilibrium between GSTO1 and GSTO2. The two polymorphisms are located only 7.5 kb apart on chromosome 10q24.3 [30] and in our association analyses we consistently estimated very similar effect sizes for both SNPs.

All calculations were performed on the entire PEG population and Caucasian subjects only (n=235 cases, 220 controls), to address the potential for population stratification. We used Mendel 6.01 software to perform linkage disequilibrium analysis, and used SAS 8.0 software to perform all other analyses.

Cases and controls were in Hardy Weinberg Equilibrium for all GST single nucleotide polymorphisms (p=0.21–0.92). Table 2 shows the genotype frequencies and associated odds ratios for Caucasian cases and controls. We noted a 32% risk reduction among Caucasian subjects carrying one or more GSTO1 variant allele (OR=0.68, 95% CI: 0.47–0.98). Similarly for GSTO2, we also observed a protective association for subjects carrying at least one variant allele. For all races together, associations were very similar (GSTO1 OR=0.76, 95% CI: 0.54–1.06; GSTO2 OR=0.68, 95% CI: 0.48–0.95). GSTO1 and GSTO2 were found to be in strong linkage disequilibrium ($p<1x10^{-6}$). No main effects were noted for the remaining polymorphisms. Stratified analyses suggested a somewhat stronger effect of the GSTO2 variant among Caucasian females although the 95% confidence intervals largely overlapped (females: OR=0.56, 95% CI: 0.32–0.98; males: OR=0.73, 95% CI: 0.44–1.21); a similar effect was noted for GSTO1. We observed no further differences in effect estimates within subgroups of gender and age at diagnosis.

Table 3 presents results for the interaction analyses. For GSTO1, we observed a large 64% risk reduction among ever regular smokers, comparing smokers with at least one variant allele to non-smoking subjects with the homozygote wildtype genotype; this departure from multiplicativity suggests an interaction between GSTO1 and smoking ($OR_{interaction}$=0.55, 95% CI: 0.33–0.92). GSTO2 presented similar results. Although we noted no main effects for carriers of the GSTM1 null genotype, we observed a 43% risk reduction among smokers with the null genotype compared to a smaller 28% reduction in risk in smokers with the gene present; however, the 95% CIs for smokers overlapped largely. Results for the remaining polymorphisms indicated no interaction on the multiplicative scale (GSTP1-105 $OR_{interaction}$ =1.35, 95% CI: 0.80–2.27; GSTP1-114 $OR_{interaction}$=1.46, 95% CI: 0.73–2.89; GSTT1 $OR_{interaction}$=0.95, 95% CI: 0.53–1.69). Results were similar when restricting analyses to subjects without a family history of PD.

Carriers of either of the two GST omega class polymorphisms were found to be strongly protected against PD in our study. GSTO1 and GSTO2, like other GSTs, have been attributed a significant function in protecting against oxidative stress [19]. The encoded proteins act as small stress response proteins in mice, and are likely involved in cellular redox homeostasis [6]. Yet the detoxification function of the GSTOs may not completely explain their protective role in PD [9]. Neuroinflammation contributes to PD pathogenesis [9,24] and studies suggest that GSTO1 may protect against neuroinflammation by modifying an inflammatory compound called interleukin-1β [14], yet the function of the GSTO1 SNP is largely unknown. However, if the GSTO1 SNP increases gene expression, GSTO1 modification of neuroinflammation might account for the observed protection against PD.

We also observed a novel multiplicative interaction between omega class GSTs and smoking. Cigarette smoking is associated with a decreased risk in PD, perhaps due to nicotine [21]. Given a recent finding that nicotine inhibits the proinflammatory compound TNFα [5], and that GSTO1 modifies the proinflammatory compound IL-1β [14], perhaps GSTO1 and some compounds in cigarette smoke both perform neuroprotective functions through modifications in the neuroinflammatory pathway.

There have been few studies examining the relationship between GSTO1, GSTO2 and PD. Whitbread (2004) did not find an association between GSTO1 and PD in a non-population based case control study in an Australian population [29]. However, GSTO1 and GSTO2 polymorphisms were found to modify age of onset of PD as well as Alzheimer's disease in a study evaluating 174 families affected with PD [15].

In agreement with previous literature, including a meta-analysis by Tan et al., we observed no independent effect for the GSTM1 homozygous gene deletion [27]. The two previous studies that addressed the association between GSTM1 and cigarette smoking have presented inconclusive results [3,4]. An early study of 100 PD cases and 200 controls reported that the protective effect of cigarette smoking was lost for patients with the GSTM1 deletion [4]. Deng used a case-only approach to examine GSTM1 and reported no departure from multiplicative interaction between smoking and GSTM1 [3]. The GSTM1 null genotype expresses no enzyme activity; one could speculate that the loss of enzyme activity may enhance the protective effect conferred by a metabolite of cigarette smoke that is not metabolized due to lack of this enzyme. Accordingly, we noted a larger effect estimate for smokers with the homozygous deletion compared to smokers without the deletion, yet the confidence intervals largely overlapped due to the small sample sizes of the subgroups and the interaction analyses indicated no departure from mulitplicativity.

The possibility of selection bias is of concern in case-control studies. Although self-selection is unlikely to be related to genotype, selection factors related to environmental risk factors

could bias estimates of main gene polymorphism effects. However, the gene-environment interaction estimates should not be influenced under the assumption that genotype does not influence participation conditional on exposure and disease, even if selection is jointly influenced by exposures and disease and whether or not the genotype is related to exposure, disease, or both [18].

We have attempted to minimize the number of comparisons by using *a priori* hypotheses. Thus, we selected only those genes for which a role in both PD and cigarette-smoke metabolism is biologically plausible and we reported all tests performed. Additionally, the polymorphisms identified as being associated with PD may in fact be in linkage disequilibrium with unidentified genes, and thus our findings may not represent causal associations.

Our determination of a multiplicative interaction between smoking and GSTO1, and GSTO2, and the paucity of similar studies, suggests a need to evaluate GST polymorphisms in conjunction with environmental toxins, as it is likely that PD is a multifactorial disease brought on by the interplay of both genetic and environmental risk factors. Further inquiry into the association between GSTs, smoking, and PD risk, and an elucidation of which chemicals in cigarette smoke serve as metabolic substrates for these GSTs may help us determine which if any substances in cigarette smoke or its metabolites are neuroprotective.

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Table 1

Demographic Characteristics of study population

	Cases n=289		Controls n=270	
	No.	**%**	**No.**	**%**
Gender				
Female	133	46.0	131	48.5
Male	156	54.0	139	51.5
Age (median (range))	70 (34–87)		69 (35–92)	
Family History PD[1]				
No	253	87.5	240	88.9
Yes	36	12.5	30	11.1
Race/ethnicity				
White	235	81.3	220	81.5
Black	3	1.0	9	3.3
Latino	32	11.1	19	7.0
Asian	4	1.4	11	4.1
Native American	15	5.2	11	4.1
Smoking Packyears[2]				
0	153	52.9	111	42.4
>0 to <10	59	20.4	65	24.3
≥10 to <40	51	17.7	60	22.4
≥40	26	9.0	32	11.9

[1] Defined as having a first degree relative with Parkinson's disease

[2] Missing packyear data for 2 smokers (n=2)

Table 2

GST genotype frequencies and adjusted odds ratios: Caucasians

	Case n=235		Control n=220		Odds Ratio	95 % Confidence Interval
	No.	%	No.	%		
GSTP1-104 (rs947894)						
AA	100	42.6%	105	47.7%	1	ref
AG	110	46.8%	90	40.9%	1.29	(0.87–1.90)
GG	25	10.6%	25	11.4%	1.04	(0.56–1.94)
AG+GG	135	57.5%	115	52.3%	1.23	(0.85–1.79)
GSTP1-114 (rs1799811)						
CC	188	80.0%	186	84.5%	1	Ref
CT	45	19.1%	31	14.1%	1.46	(0.88–2.41)
TT	2	0.9%	3	1.4%	0.65	(0.11–3.93
CT+TT	47	20.0%	34	15.5%	1.38	(0.85–2.25
GSTO1-140 (rs4925)						
CC	125	53.2%	96	43.6%	1	Ref
AC	93	39.6%	101	45.9%	0.70	(0.48–1.04)
AA	17	7.2%	23	10.5%	0.56	(0.28–1.11
AC+AA	110	46.8%	124	56.4%	0.68	(0.47–0.98
GSTO2 (rs2297235)						
AA	126	53.6%	94	42.7%	1	Ref
AG	90	38.3%	106	48.1%	0.63	(0.43–0.93)
GG	19	8.1%	20	9.1%	0.69	(0.35–1.38)
AG+GG	109	46.4%	126	57.3%	0.64	(0.44–0.93)
GSTM1[1]						
*1	121	51.5%	114	51.8%	1	Ref
*0	114	48.5%	106	48.2%	1.00	(0.69–1.45)
GSTT1[1]						
*1	165	70.2%	162	73.6%	1	Ref
*0	70	29.8%	58	26.4%	1.19	(0.79–1.78)

OR, odds ratio adjusted for sex and age at diagnosis (continuous)

[1] *0 signifies homozygous gene deletion

Table 3

Interaction analysis between smoking and GSTO1, GSTO2, and GSTM1: Caucasians

Polymorphism	Never-Regular Smoked (n=219)			Ever-Regular Smoked (n=236)[1]		
	Cases/ Ctrls	OR	95% CI	Cases/ Ctrls	OR	95% Confidence Interval
GSTO1-140 (rs4925)						
CC	61/38	1	ref	64/58	0.66	(0.38–1.14)
AC+AA	67/53	0.77	(0.44–1.32)	43/71	0.36	(0.21–0.63)
Interaction OR					*0.55*	*(0.33–0.92)*
GSTO2 (rs2297235)						
AA	63/38	1	ref	63/56	0.65	(0.37–1.12)
AG+GG	65/53	0.72	(0.42–1.25)	44/73	0.35	(0.20–0.61)
Interaction OR					*0.54*	*(0.32–0.90)*
GSTM1[2]						
*1	61/49	1	ref	60/65	0.72	(0.43–1.21)
*0	67/42	1.28	(0.74–2.21)	47/64	0.57	(0.33–0.97)
Interaction OR					*0.78*	*(0.47–1.31)*

OR, odds ratio adjusted for sex and age at diagnosis (continuous)

[1] Ever-regular smoked is defined as smoking cigarettes for at least one year.

[2] *0 signifies homozygous gene deletion