Glutathione S-transferase M1, T1, and P1 Polymorphisms and Parkinson's Disease

Samir N. Kelada[a], Patricia L. Stapleton[a], Federico M. Farin[a], Theo K. Bammler[a], David L. Eaton, Terri Smith-Weller[a], Gary M. Franklin[a,b], Phillip D. Swanson[b], W.T. Longstreth Jr[b,c], Harvey Checkoway[a,c,*]

[a]Department of Environmental Health, Box 357234, University of Washington, Seattle, WA 98195-7234, USA
[b]Department of Neurology, University of Washington, Seattle, WA 98195-6465, USA
[c]Department of Epidemiology, University of Washington, Seattle, WA 98195-7236, USA

Abstract

Oxidative stress is widely thought to contribute significantly to the pathogenesis Parkinson's disease (PD). Given the role of glutathione S-transferases (GSTs) in the conjugation of electrophiles and protection against reactive oxygen species, genes encoding the GSTs have been considered candidates for association studies of PD. We tested for associations between genotypes of *GSTM1*(homozygous deletion vs. non-deleted), *GSTT1*(homozygous deletion vs. non-deleted), and *GSTP1* (Ile104Val and Ala113Val) and PD in a case-control study of 214 idiopathic PD cases and 330 age- and gender-matched, unrelated controls of Caucasian ethnicity. No significant associations with any of the *GST* genotypes were observed. However, there was a marginally significant difference in the distribution of *GSTP1* 104 genotypes between cases and controls ($P = 0.07$), with an excess of Ile104Val heterozygotes found among cases (odds ratio (OR) = 1.43; 95% Confidence Interval (CI): 0.98–2.08). This difference in the genotype distribution was strongest among smokers (OR for heterozygote = 1.92; 95% CI: 1.12–3.29) versus non-smokers and among males (OR for heterozygote = 1.99; 95% CI: 1.24–3.19) versus females. The distribution of *GSTP1* Ile104Val and Ala113Val haplotypes did not differ between cases and controls. Taken together, these results suggest a potentially minor role of *GSTP1* in PD, but do not give evidence for associations with either *GSTM1* or *GSTT1*.
© 2002 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Parkinson's disease; Glutathione S-transferase; Oxidative stress; Epidemiology

Oxidative stress in dopaminergic pathways is thought to be of central importance in the pathogenesis of Parkinson's disease (PD) [14]. Postmortem studies of PD brains demonstrate increased indices of oxidative stress, including increased levels of iron, increased lipid peroxidation, decreased mitchondrial complex I activity, and decreased levels of glutathione in the substantia nigra [9,10]. Furthermore, reactive oxygen species generated by the oxidation of dopamine contribute to the dopaminergic cytotoxicity induced by α-synuclein, a major Lewy body protein [7,17,18].

Glutathione S-transferases (GSTs) are dimeric, cytosolic enzymes that protect against oxidative stress by conjugating glutathione to electrophilic species that can adduct protein or DNA and generate reactive oxygen species. Because of this important role in cellular defense against oxidative stress, GSTs have been considered candidates for association studies with PD. The genes encoding three classes of GSTs, GSTM1 (mu, chromosome 1p13.3), GSTP1 (pi, chromosome 11q13), and GSTT1 (theta, chromosome 22q11.2), are known to be polymorphic. Homozygous deletion genotypes of *GSTM1* and *GSTT1* are relatively common in Caucasian populations (~50% and 15–20%, respectively) [6]. Single nucleotide polymorphisms in *GSTP1*, resulting in amino acid substitutions at codons 104 (Ile → Val) and 113 (Ala → Val) are also relatively frequent in the Caucasian population [6].

Previous case-control studies of *GST* polymorphisms and PD have yielded mixed results. Stroombergen and Waring [15] observed increased frequencies of homozygous deleted *GSTM1* and *GSTT1* genotypes among PD cases compared to

controls. Similarly, De Palma et al. [5] reported an increased frequency of homozygous deleted *GSTT1* genotypes among PD cases. In contrast, other studies found no significant differences in *GSTM1* and *GSTT1* genotype frequencies between PD cases and controls [1,2,8,16]. Menegon et al. [12] reported findings from another case-control study in which no difference in *GSTP1* genotype frequencies was observed between all cases and controls, although there was a difference among a subset of pesticide-exposed subjects in which the variant *GSTP1*104Val allele was more prevalent among cases than controls.

We investigated the role of *GST* polymorphisms in PD in a population-based case-control study. Newly diagnosed idiopathic PD patients ($n = 214$, aged 37–88 years, mean age (SD) = 68.0 (9.6)), were identified by neurology and general medical practice clinics of the Group Health Cooperative (GHC) from the Puget Sound area in Western Washington State. Inclusion criteria for the cases were the presence of at least two of the four cardinal signs of PD: bradykinesia, resting tremor, cogwheel rigidity, and postural reflex impairment. Exclusion criteria included the use of certain medications during the 12 months preceding symptom onset, prior history of multiple cerebrovascular events, or another explanation for parkinsonism symptoms (e.g., brain injury, brain tumor, encephalitis). To verify PD diagnoses, three study neurologists (P.D.S., G.M.F., and W.T.L.) reviewed charts for cases not referred by GHC neurologists.

Control subjects ($n = 330$, aged 44–85 years, mean age (SD) = 69.3 (8.6)) were identified from GHC enrollees without past histories of PD or other neurodegenerative disorders. Controls were matched to cases by birth decade, gender, and year of enrollment in GHC. 60.7% of cases and 64.2% of controls were male. All subjects were of non-Hispanic Caucasian ethnicity. Subjects were considered as 'ever smokers' if they had smoked at least 100 cigarettes in their lifetime, based on responses to an in-person questionnaire. Study subjects were volunteers who were informed of the purpose of the study. The Institutional Review Board committees on Human Subjects Research at the University of Washington and GHC Center for Health Studies approved study forms and procedures.

DNA was extracted from whole blood using standard techniques. *GSTM1* and *GSTT1* genotypes were then determined by polymerase chain reaction (PCR) using known primers and conditions [4]. TaqMan™ Detection System-based assays were developed to identify the *GSTP1* genotypes [11]. The PCR primers and the TaqMan™ probe for these genotyping assays were designed using the primer design software Primer Express™ (Perkin-Elmer Applied Biosystems). Synthesis of probes was performed by MWG Biotech, Inc. (High Point, North Carolina). For *GSTP1* Ile104Val genotype, the forward and reverse primers were 5′-GGCAGCCCTGGTGGACAT-3′ and 5′-AGCCCC-CAGTGCCCAAC-3′, respectively. The wild-type probe used was 5′-TCCGCTGCAAATACATCTCCCTCATC-

TAC-3′, and was 5′ labeled with 6FAM™ fluorescent dye and 3′ conjugated with TAMRA™ quencher dye. The mutant probe, 5′-CCGCTGCAAATACGTCTCCCT-CATCTA-3′, was 5′ labeled with VIC™ fluorescent dye and 3′ conjugated with TAMRA™ quencher dye. Cycling conditions for the reaction were initial denaturation at 95 °C for 2:00 followed by 41 cycles of denaturation at 95 °C for 30 s and extension at 67 °C for 40 s. Similarly, for *GSTP1* Ala113Val genotyping, the forward and reverse primers were 5′-GGAGGGATGAGAGTAGGATGATACAT-3′ and 5′-GGGACAGCAGGGTCTCAAAA-3′, respectively. The wild-type and mutant probes, respectively, were 5′-CCTTGCCCGCCTCCTGCC-3′, 5′ labeled with 6FAM™ fluorescent dye and 3′ conjugated with TAMRA™ quencher dye, and 5′-CATCCTTGCCCACCTCCTGCC-3′, 5′ labeled with TET™ fluorescent dye and 3′ conjugated with TAMRA™ quencher dye. Cycling conditions for the reaction were initial denaturation at 95 °C for 2:00 followed by 40 cycles of denaturation at 95 °C for 20 s and extension at 67 °C for 40 s. Associations between genotypes and PD were examined with chi square tests and calculation of age- and gender-adjusted odds ratios (OR) by logistic regression.

The distributions of *GST* genotypes among cases and controls are shown in Table 1. No associations were found with the deletion genotypes of either *GSTM1* or *T1*. A marginally significant difference in the distribution of *GSTP1* Ile104Val genotypes was observed ($\chi^2 = 5.27$, $P = 0.07$), with Ile104Val heterozygotes having a small increase in PD risk (adjusted OR = 1.43; 95% Confidence Interval (CI): 0.98–2.08). Curiously, no increase in risk was observed with the homozygous 104Val genotype (OR = 0.86; 95% CI: 0.50–1.47). *GSTP1*104Ile allele frequencies were 0.62 and 0.63 in cases and controls, respectively ($\chi^2 = 0.08$, $P = 0.77$). *GSTP1* 113Ala allele frequencies did not differ significantly between cases (113Ala allele frequency = 0.90) and controls (113Ala allele frequency = 0.89; $\chi^2 = 0.27$, $P = 0.60$). The distribution of *GSTP1* Ala113Val genotypes was slightly different between cases and controls though ($\chi^2 = 4.88$, $P = 0.09$); however no increased risk was associated with the heterozygote genotype. There were insufficient numbers of study subjects with the homozygous 113Val genotype to allow for a measure of association.

A significant interaction between *GSTP1* Ile104Val genotype and gender was found (likelihood ratio test $\chi^2 = 7.48$, 2 d.f., $P = 0.02$). Subsequent stratified analysis of *GSTP1* Ile104Val genotype distributions by gender (Table 2A) showed that the difference was stronger in males ($\chi^2 = 12.22, P = 0.002$ vs. $\chi^2 = 0.51, P = 0.78$ for females). The OR for male *GSTP1*Ile104Val heterozygotes was 1.99 (95% CI: 1.12–3.29), although the homozygous 104Val genotype was again not associated with increased risk. Differences in the *GSTP1* Ile104Val genotype distribution between cases and controls stratified by age (<60 versus ≥60 years of age) were also observed, although the test for interaction was not significant (likelihood ratio test $\chi^2 = 1.14$, 2 d.f., $P = 0.57$).

Table 1
Associations of *GSTM1*, *T1*, and *P1* genotypes and Parkinson's disease[a]

Gene	Cases (%)	Controls (%)	Genotype Distribution χ^2	OR (95% CI)	OR_{adj}* (95% CI)
GSTM1					
Present	106 (49.5)	178 (54.4)		1.0 (referent)	1.0 (referent)
Null	108 (50.5)	149 (45.6)	1.25, $P = 0.26$	1.22 (0.86–1.72)	1.20 (0.85–1.70)
GSTT1					
Present	176 (82.2)	261 (79.8)		1.0 (referent)	1.0 (referent)
Null	38 (17.8)	66 (20.2)	0.49, $P = 0.48$	0.85 (0.55–1.33)	0.85 (0.54–1.32)
GSTP1 104					
Ile/Ile	79 (37.1)	141 (42.9)		1.0 (referent)	1.0 (referent)
Ile/Val	107 (50.2)	133 (40.4)		1.44 (0.98–2.09)	1.43 (0.98–2.08)
Val/Val	27 (12.7)	55 (16.7)	5.27, $P = 0.07$	0.88 (0.63–1.50)	0.86 (0.50–1.47)
GSTP1 113					
Ala/Ala	170 (79.8)	263 (79.9)		1.0 (referent)	1.0 (referent)
Ala/Val	43 (20.2)	59 (17.9)		1.13 (0.73–1.75)	1.13 (0.73–1.75)
Val/Val	0 (0.0)	7 (2.1)	4.88, $P = 0.09$	–	–

[a] *Adjusted for age ($<$60, $\geq$60) and gender.

The difference in the distribution was stronger among older subjects ($\chi^2 = 5.22$, $P = 0.07$) than among younger subjects ($\chi^2 = 1.21$, $P = 0.55$). Among the older subjects, the OR for the heterozygote genotype was 1.52 (95% CI: 1.01–2.30), as shown in Table 2B.

Given the well-documented protective effect of smoking in PD [3,13], we also assessed interactions between *GSTP1* Ile104Val genotype and smoking status (ever vs. never). Ever smoking was associated with reduced PD risk (OR = 0.49; 95% CI: 0.34–0.71), and the interaction with *GSTP1* 104 genotype was suggestive but not significant (likelihood ratio test $\chi^2 = 4.08$, 2 d.f., $P = 0.13$). The smoking-stratification genotype distributions and ORs are shown in Table 2C. Heterozygotes were more prevalent among cases who smoked (OR = 1.92, 95% CI: 1.12–3.29; genotype distribution $\chi^2 = 8.64$, $P = 0.01$) than among non-smokers (OR = 1.04; 95% CI: 0.60–1.83; $\chi^2 = 0.05$, $P = 0.98$).

Finally, we analyzed *GSTP1* Ile104Val and Ala113Val haplotype frequencies among cases and controls, as shown in Table 3. Our analysis showed that the two regions are in tight linkage disequilibrium ($P < 0.001$), as was expected given the close proximity of these two polymorphisms. No differences in haplotype frequencies between cases and controls were found.

To our knowledge, this is the largest case-control study to have investigated associations between PD and *GSTM1*, *GSTT1*, and *GSTP1* genotypes. Overall, the results do not

Table 2
Stratified ORs for *GSTP1* Ile104Val genotype[a]

(A)	Men		Women	
GSTP1 104 genotype	$N_{cases}/N_{controls}$	OR_{adj}* (95% CI)	$N_{cases}/N_{controls}$	OR_{adj}* (95% CI)
Ile/Ile	47/101	1.0 (referent)	32/40	1.0 (referent)
Ile/Val	71/77	1.99 (1.24–3.19)	36/56	0.79 (0.42–1.48)
Val/Val	11/34	0.70 (0.33–1.50)	16/21	0.95 (0.43–2.12)
Genotype distribution χ^2	12.22 $P = 0.002$		0.51 $P = 0.78$	
(B)	Age $<$ 60		Age $\geq$ 60	
GSTP1 104 genotype	$N_{cases}/N_{controls}$	OR_{adj}** (95% CI)	$N_{cases}/N_{controls}$	OR_{adj}** (95% CI)
Ile/Ile	16/20	1.0 (referent)	63/121	1.0 (referent)
Ile/Val	16/20	1.00 (0.39–2.54)	91/113	1.52 (1.01–2.30)
Val/Val	4/10	0.53 (0.14–2.02)	23/45	0.95 (0.53–1.72)
Genotype distribution χ^2	1.21 $P = 0.55$		5.22 $P = 0.07$	
(C)	Ever smokers		Never smokers	
GSTP1 104 genotype	$N_{cases}/N_{controls}$	OR_{adj}*** (95% CI)	$N_{cases}/N_{controls}$	OR_{adj}*** (95% CI)
Ile/Ile	33/92	1.0 (referent)	43/49	1.0 (referent)
Ile/Val	52/78	1.92 (1.12–3.29)	51/55	1.04 (0.60–1.83)
Val/Val	9/36	0.69 (0.30–1.60)	17/18	1.05 (0.48–2.32)
Genotype distribution χ^2	8.64 $P = 0.01$		0.05 $P = 0.98$	

[a] *OR adjusted for age ($<$60, $\geq$60), **OR adjusted for gender, ***OR adjusted for age ($<$60, $\geq$60) and gender.

Table 3
Haplotype frequencies among Parkinson's disease cases and controls[a]

SNP		Frequency (%)	
104	113	Cases ($n = 213$)	Controls ($n = 329$)
Ile	Ala	132 (62.2)	205 (62.3)
Ile	Val	0 (0.0)	3 (0.8)
Val	Ala	59 (27.7)	87 (26.6)
Val	Val	22 (10.1)	34 (10.3)

[a] Test statistic for difference in haplotype frequency between cases and controls: $\chi^2 = 3.88$, three degrees of freedom, $P = 0.27$.

suggest that *GSTM1* or *GSTT1* genotypes are related to PD. *GSTP1* genotype, however, may have a minor role, as evidenced by the mildly elevated OR for Ile104Val heterozygotes. The increased prevalence of Ile104Val heterozygotes found here is similar to that observed by Menegon et al. [12] among their pesticide-exposed case-control subjects. Our results suggest that decreased conjugation of some GST-pi substrate(s) may be relevant to the etiology of PD. Specific relevant substrates remain to be identified, but some initial clues may come from our observation that the association between the Ile104Val genotype and PD was stronger among males than females, subjects ≥ 60 years of age vs. <60, and among smokers vs. non-smokers. The lack of an association between the homozygous 104Val genotype, however, does not strengthen the hypothesis that the *GSTP1* 104Val allele confers increased PD risk.

[1] Ahmadi, A., Fredrikson, M., Jerregard, H., Akerback, A., Fall, P.A., Rannug, A., Axelson, O. and Soderkvist, P., GSTM1 and mEPHX polymorphisms in Parkinson's disease and age of onset, Biochem. Biophys. Res. Commun., 269 (2000) 676–680.

[2] Bandmann, O., Vaughan, J., Holmans, P., Marsden, C.D. and Wood, N.W., Association of slow acetylator genotype for N-acetyltransferase 2 with familial Parkinson's disease, Lancet, 350 (1997) 1136–1139.

[3] Checkoway, H., Powers, K., Smith-Weller, T., Franklin, G.M., Longstreth Jr, W.T. and Swanson, P.D., Parkinson's disease risks associated with cigarette smoking, alcohol consumption, and caffeine intake, Am. J. Epidemiol., 155 (2002) 732–738.

[4] Chen, C.L., Liu, Q. and Relling, M.V., Simultaneous characterization of glutathione S-transferase M1 and T1 polymorphisms by polymerase chain reaction in American whites and blacks, Pharmacogenetics, 6 (1996) 187–191.

[5] De Palma, G., Mozzoni, P., Mutti, A., Calzetti, S. and Negrotti, A., Case-control study of interactions between genetic and environmental factors in Parkinson's disease, Lancet, 352 (1998) 1986–1987.

[6] Garte, S., Gaspari, L., Alexandrie, A.K., Ambrosone, C., Autrup, H., Autrup, J.L., Baranova, H., Bathum, L., Benhamou, S., Boffetta, P., Bouchardy, C., Breskvar, K., Brockmoller, J., Cascorbi, I., Clapper, M.L., Coutelle, C., Daly, A., Dell'Omo, M., Dolzan, V., Dresler, C.M., Fryer, A., Haugen, A., Hein, D.W., Hildesheim, A., Hirvonen, A., Hsieh, L.L., Ingelman-Sundberg, M., Kalina, I., Kang, D., Kihara, M., Kiyohara, C., Kremers, P., Lazarus, P., Le Marchand, L., Lechner, M.C., van Lieshout, E.M., London, S., Manni, J.J., Maugard, C.M., Morita, S., Nazar-Stewart, V., Noda, K., Oda, Y., Parl, F.F., Pastorelli, R., Persson, I., Peters, W.H., Rannug, A., Rebbeck, T., Risch, A., Roelandt, L., Romkes, M., Ryberg, D., Salagovic, J., Schoket, B., Seidegard, J., Shields, P.G., Sim, E., Sinnet, D., Strange, R.C., Stucker, I., Sugimura, H., To-Figueras, J., Vineis, P., Yu, M.C. and Taioli, E., Metabolic gene polymorphism frequencies in control populations, Cancer Epidemiol. Biomark. Prev., 10 (2001) 1239–1248.

[7] Giasson, B.I., Duda, J.E., Murray, I.V., Chen, Q., Souza, J.M., Hurtig, H.I., Ischiropoulos, H., Trojanowski, J.Q. and Lee, V.M., Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions, Science, 290 (2000) 985–989.

[8] Harada, S., Fujii, C., Hayashi, A. and Ohkoshi, N., An association between idiopathic Parkinson's disease and polymorphisms of phase II detoxification enzymes: glutathione S-transferase M1 and quinone oxidoreductase 1 and 2, Biochem. Biophys. Res.Commun., 288 (2001) 887–892.

[9] Hirsch, E.C., Brandel, J.P., Galle, P., Javoy-Agid, F. and Agid, Y., Iron and aluminum increase in the substantia nigra of patients with Parkinson's disease: an X-ray microanalysis, J. Neurochem., 56 (1991) 446–451.

[10] Jenner, P., Altered mitochondrial function, iron metabolism and glutathione levels in Parkinson's disease, Acta Neurol. Scand. Suppl., 146 (1993) 6–13.

[11] Livak, K.J., Flood, S.J., Marmaro, J., Giusti, W. and Deetz, K., Oligonucleotides with fluorescent dyes at opposite ends provide a quenched probe system useful for detecting PCR product and nucleic acid hybridization, PCR Methods Appl., 4 (1995) 357–362.

[12] Menegon, A., Board, P.G., Blackburn, A.C., Mellick, G.D. and Le Couteur, D.G., Parkinson's disease, pesticides, and glutathione transferase polymorphisms, Lancet, 352 (1998) 1344–1346.

[13] Morens, D.M., Grandinetti, A., Reed, D., White, L.R. and Ross, G.W., Cigarette smoking and protection from Parkinson's disease: false association or etiologic clue? Neurology, 45 (1995) 1041–1051.

[14] Olanow, C.W. and Tatton, W.G., Etiology and pathogenesis of Parkinson's disease, Annu. Rev. Neurosci., 22 (1999) 123–144.

[15] Stroombergen, M.C. and Waring, R.H., Determination of glutathione S-transferase mu and theta polymorphisms in neurological disease, Hum. Exp. Toxicol., 18 (1999) 141–145.

[16] Tison, F., Coutelle, C., Henry, P. and Cassaigne, A., Glutathion S-transferase (class mu) phenotype in Parkinson's disease, Mov. Disord., 9 (1994) 117–118.

[17] Xu, J., Kao, S.Y., Lee, F.J., Song, W., Jin, L.W. and Yankner, B.A., Dopamine-dependent neurotoxicity of alpha-synuclein: a mechanism for selective neurodegeneration in Parkinson disease, Nat. Med., 8 (2002) 600–606.

[18] Zhou, W., Hurlbert, M.S., Schaack, J., Prasad, K.N. and Freed, C.R., Overexpression of human alpha-synuclein causes dopamine neuron death in rat primary culture and immortalized mesencephalon-derived cells, Brain Res., 866 (2000) 33–43.