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Association of GST M1 null polymorphism with Parkinson's disease in a Chilean population with a strong Amerindian genetic component

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Abstract

We have studied the association of a null mutation of Glutathione Transferase M1 (GST M1*0/0) with Parkinson's disease (MIM 168600) in a Chilean population with a strong Amerindian genetic component. We determined the genotype in 349 patients with idiopathic Parkinson's disease (174 female and 175 male; 66.84 ± 10.7 years of age), and compared that to 611 controls (457 female and 254 male; 62 ± 13.4 years of age). A significant association of the null mutation in GST M1 with Parkinson's disease was found ($p = 0.021$), and the association was strongest in the earlier age range. An association of GSTM1*0/0 with Parkinson's disease supports the hypothesis that Glutathione Transferase M1 plays a role in protecting astrocytes against toxic dopamine oxidative metabolism, and most likely by preventing toxic one-electron reduction of aminochrome.
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Parkinson's disease (PD) is a neurodegenerative disorder characterized by degeneration of neuromelanin-/dopamine-containing neurons of the nigrostriatal tract. A number of gene mutations and deletions have been reported to play a role in the pathogenesis of familial PD. Moreover, a number of pathological and pharmacological studies on sporadic PD and dopaminergic neurotoxin-induced parkinsonism have hypothesized that mitochondrial dysfunction, inflammation, oxidative stress, and dysfunction of the ubiquitin-proteasome system all play important roles in the pathogenesis and progression of PD [1,13]. However, these hypotheses do not yet fully explain the mechanisms associated with dopaminergic neuron-specific cell loss in PD, since the cause of nigral dopaminergic neuronal degeneration in PD remains unknown. Several lines of evidence indicate that dopamine oxidation plays a role in the degeneration of neuromelanin-containing neurons in PD [27]. Dopamine is known to induce toxicity and apoptosis in vitro in a number of cells including neuronal cell lines [11,28]. One-electron reduction of aminochrome has been proposed as the major source for endogenous generation of reactive species involved in the degenerative process leading to dopamine neuronal cell death and eventual progression to PD [4,26,22,3,23]. The dopamine oxidation product, aminochrome, forms adducts with alpha-synuclein, giving rise to alpha-synuclein protofibrils and thereby inhibiting alpha-synuclein fibrillization [oligomerization] into protofibrils, which represent the crucial element in the genesis of dopaminergic neuropathy [8,20,21].

One-electron reduction of aminochrome can also be prevented by GST M2 and GST M1 [25,5,15]. Null phenotypes for GSTM1 with a deletion in GSTM1 gene [GSTM1*0/0] have been described, resulting in the lack of expression of the enzyme. The existence of null polymorphism in the GST M1 gene, which

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prevents aminochrome toxicity, gives rise to the question regarding the possible association of this polymorphism with PD. In this study, we investigated the possible association of GSTM1 null mutation with PD in 349 patients and in 611 controls with a strong Amerindian genetic component.

Taq DNA polymerase was obtained from Life Technologies [California, USA). EZNA blood DNA kit was from Omega Bio-Tek (USA). The primers were obtained from T-A-G-Copenhagen A/S (Copenhagen, Denmark).

A total of 349 patients (174 females and 175 males) with idiopathic PD and 611 controls (457 females and 254 males) were recruited from Santiago (90%) and Puchuncavi (10%). The majority of patients were recruited from Parkinson's Liga, which is a patients' organization or a group of patients of those neurologists participating in this study. The controls were volunteers recruited from the same geographic areas by announcements published in seniors' organizations or by local radio ads. In Chile, the Amerindian genetic component correlates with the socioeconomic level: it is small (typically less than 10%) in the upper strata, and greater in lower strata (about 40%) [30]. Santiago is a city of about 5–6 million inhabitants with a heterogeneous population, and with areas of marked European influence with high economical and educational level. There are also areas with a majority of native genetic influence with low economical and educational level. Puchuncavi is a rural area located close to the Pacific Ocean, 140 km north of Santiago, and with 13,000 habitants which comprise a native population with low economic and educational level. Blood samples collected respectively from controls (84%) and Parkinson's patients (82%) are thus derived from a population with low economic and educational level, and with strong native features. Control samples were recruited from healthy volunteers, who underwent an examination by an experimented neurologist, in order to certify that these subjects did not have PD or another neurological disorder. Patients or control subjects presenting other neurological disorders were not included in this study. All patients in this study were specifically diagnosed by neurologists and confirmed as having idiopathic PD, presenting at least two of four cardinal signs: bradykinesia, rest tremor, rigidity, and postural reflex impairment group. Exclusion criteria for a diagnosis of idiopathic PD disease were the use of medications (e.g., phenothiazines) during the 12 months preceding symptom onset; MRI or CT evidence of multiple cerebrovascular events prior to symptom onset; evidence of another known cause of parkinsonism (e.g., history of brain tumor or encephalitis); or atypical PD presentation. Ethical approval of this study was obtained from the Ethical Committee of the Faculty of Medicine, University of Chile. All study subjects including controls and Parkinson's patients gave informed consent for providing questionnaire data and blood samples for DNA extraction. We declare that all experiments on human subjects were conducted in accordance with the Declaration of Helsinki (http://www.wma.net).

Blood samples from 349 patients and 611 controls were collected, and genomic DNA was extracted using the Genomic Prep DNA Isolation Kit (Amersham Pharmacia Biotech) and following the manufacturer's instructions.

The polymorphic deletion of the GSTM1 (null genotype) gene was determined by using PCR. Two hundred nanograms of genomic DNA were amplified to a fragment of 272 bp by using a specific primer for GST M1, forward 5′CTGCCCTACTTGATTG ATGGG′3 and reverse 5′CTGGATTGTAGCAGATCATGC′3 (accession number X68676). As internal standards we amplified a fragment of 170 bp of interferon using specific primers, forward 5′GGCACAACAGGTAGTAGGCG′3 and reverse 5′GCCACAGGAGCTTCTGA CAC′3. The incubation mixture contained 3 mM $MgCl_2$, 0.5 μM primers, 0.2 mM dNTP, and 1 U taq polymerase. The PCR conditions were 5 min at 95 °C, 35 cycles (30 s at 95 °C, 40 s at 60 °C and 1 min 72 °C) and 10 min at 72 °C. This method allowed us to determine the normal phenotype by showing the GSTM1 band and the band for interferon used as internal standard. However, this method showed only the homozygote null genotype GSTM1 (GSTM1*0/GSTM1*0) where the band for the product of GSTM1 is lacking, and in which we could observe the presence of a band of interferon. Therefore, the heterozygote genotype for the null mutation (GSTM1*0/GSTM1*A(B)) could not be determined with this method; the heterozygote genotype was included in the results of the normal genotype. The amplifications were carried out in triplicate and some of them were verified by sequencing (10 randomly selected samples).

Due to the method utilized to determine a null mutation in GST M1, it was not possible to calculate the allele frequencies of the enzyme. Therefore, we merely calculated genotypes frequencies. Odds ratio (OR) and its confidence interval (CI) were calculated using Epi Info version 6 package.

Included in this study were 349 patients diagnosed with idiopathic PD and 611 controls which were examined by an experienced neurologist who determined that this group did not present any symptoms of PD or other neurological disorder. Data analysis was performed using the entire population, or subgroups

Table 1
GST M1 genotypes in control and Parkinson's patients

	n	GST M1	GST M*0/0
(A) Totals, control and Parkinson's patients			
Control	611	367 (60)	244 (40)
Patients	349	185 (53)	164 (47)
(B) Total, control and Parkinson's patients younger than 45 years of age			
Controls	67	32 (48)	35 (52)
Patients	11	6 (54.5)	5 (45.5)
(C) Total, control and Parkinson's patients 45–64 years of age			
Controls	235	154 (65.5)	81 (34.5)
Patients	124	59 (47.6)	65 (52.4)
(D) Total, control and Parkinson's patients younger than 65 years of age			
Controls	302	186 (62)	116 (38)
Patients	135	65 (48)	70 (52)
(E) Total, control and Parkinson's patients 65 years of age and older			
Controls	309	181 (59)	128 (41)
Patients	214	120 (56)	94 (44)

The genotype GSTM1*0/0 corresponds to the homozygote alleles (GSTM1*0/GSTM1*0), and GSTM1 is composed of GSTM1*A, GSTM1*B, GSTM1*A/B and GSTM1*0/A(B). Percentages in parentheses.

derived by age class: younger than 45 years, between 45 and 64 years, younger than 65 years, and older than 65 years of age. These age-brackets were derived, because of the observation that the GSTM1*0/0 genotype in Parkinson's patients has a significantly reduced median age (57 years) compared with patients with the GSTM1 genotype (68 years) [2].

The results obtained for GSTM1 *loci* in 349 patients (66.8 ± 10.7 years; range, 25–93 years of age) and 611 healthy controls (62.0 ± 13.4 years; range, 15–90 years of age) are shown in Table 1A. The genotype GSTM1*0/0 for the GSTM1 locus was significantly augmented in patients ($p = 0.04$). No significant difference was observed for the loci of GSTM1 in the group younger than 45 years of age, probably due to the low number of subjects (11 patients and 67 controls) (Table 1B). However, in the group between 45 and 64 years of age, shown in Table 1C, the genotype GSTM1*0/0 for the GSTM1 locus was significantly augmented in patients ($p = 0.0009$).

In the group younger than 65 years of age (Table 1D) GSTM1*0/0 for the GSTM1 locus was significantly augmented in patients ($p = 0.0092$). Finally, the results in the groups older than 65 are shown in Table 1E. The genotype GSTM1*0/0 for the GSTM1 locus did not show a significant difference between patients and controls.

There are conflicting literature reports on possible association of the GSTM1 null mutation and PD. One study conducted in the United Kingdom found that males with a deletion of the GSTM1 gene were more susceptible to PD [29]. Conversely, studies from Japan, comparing 111 patients with idiopathic PD with 100 healthy volunteers, found no association between the GSTM1 null mutation and PD. Lack of an association between the GSTM1 null mutation and PD was also found (1) in Germany, comparing 149 PD patients with 99 control subjects; and the same; (2) for a study comparing 214 PD patients with 330 age- and gender-matched, unrelated controls of Caucasian eth-

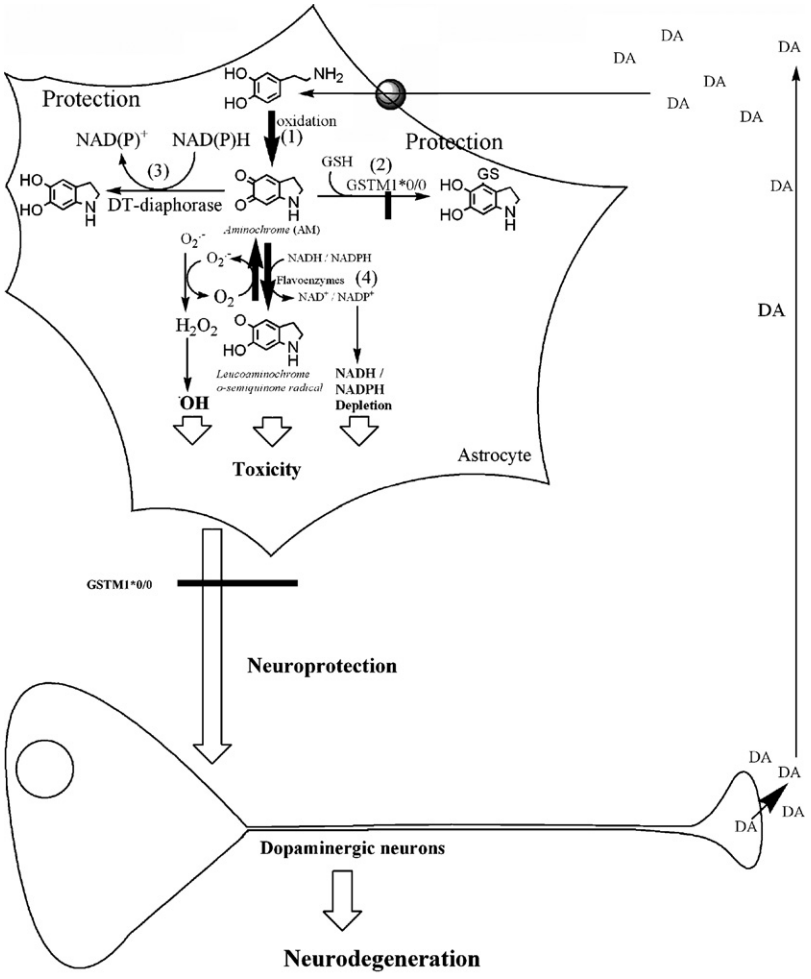

Fig. 1. Possible mechanism for the neuroprotective role of GST M1 in astrocytes. Astrocytes take up dopamine released from dopaminergic neurons. Dopamine in the cytosol autoxidizes to aminochrome at physiological pH (reaction 1). Aminochrome has three possible metabolic pathways: (i) aminochrome one-electron reduction to leukoaminochrome *o*-semiquinone radical, which is a potent endogenous neurotoxin (reaction 4). Leukoaminochrome *o*-semiquinone radical is very reactive with oxygen, generating redox cycling which depletes NADH, NADPH and oxygen—with concomitant formation of superoxide radicals; (ii) aminochrome can be reduced by two-electrons to leukoaminochrome, catalyzed by DT-diaphorase which prevents one-electron reduction of aminochrome (reaction 3); and (iii) aminochrome can be conjugated with GSH to a glutathionyl conjugate which is resistant to biological oxidizing agents such as oxygen, superoxide radicals and hydrogen peroxide (reaction 2). This is a protective reaction since it prevents aminochrome one-electron reduction.

nicity; and (3) for a study done in Australia with 400 PD patients and 402 control subjects [16,24,19,10]. However, in our Chilean study which engaged 349 patients and 611 controls with a strong Amerindian genetic component, a significant association of the GSTM1*0/0 genotype with PD was found. The discrepant findings in different countries, relating to GSTM1 and PD, may be attributable to genetic differences in the populations of these respective countries. In our study of a population with strong Amerindian genetic component, there was a strong association of the GSTM1*0/0 genotype with PD, particularly at younger ages. This finding is in agreement with a Swedish study in which the median age of onset of PD was lower in patients with the GSTM1*0/0 genotype—a median age of 57 years versus 68 years in patients with the GSTM1 gene [29].

The one-electron reduction product of the dopamine metabolite aminochrome is a hypothesized mediator of intraneuronal neurotoxic processes, proposed to be responsible for the demise of dopaminergic neurons in PD [22,3,23]. The formation of alpha-synuclein protofibrils is enhanced and stabilized by dopamine quinones (aminochrome) derived from the oxidation of dopamine [8,20,21]. Alpha-synuclein oligomerization into cytotoxic protofibrils seems to be essential for neurotoxic events [20]. GSTM1 or GSTM2 catalyzes the conjugation of aminochrome to a product which is resistant to oxidation by superoxide radicals and hydrogen peroxide [25,5]. In addition, GSTM2 catalyzes the conjugation of the precursor of aminochrome, namely dopamine *o*-quinone, to 5-glutathionyl dopamine [9], which is the precursor of 5-cysteinyl-dopamine encountered in rat, guinea pig and human brain [7]. GSTM is localized in human brain (Campbell et al. 1990) [6], while GSTM2 has been described in human substantia nigra [5], and GSTM1 has been detected in astrocytoma—suggestive of astrocytic origin [14]. GSTM1 has a protective role, preventing one-electron reduction of aminochrome, which otherwise generates a potent endogenous neurotoxin [22,3,23] proposed to be responsible for the formation of reactive oxygen species during the degenerative processes in PD. This protective effect is in agreement with the association of GSTM1*0/0 deletion with the disease, since astrocytes seem to play an important neuroprotective role for dopaminergic neurons. We propose that GSTM1 functions to protect astrocytes against aminochrome toxicity, thereby affording healthy astrocytes with a neuroprotectic action for dopaminergic neurons (Fig. 1). The protective role of astrocytes on dopaminergic neurons is supported by several lines of evidence: (i) astrocyte-mediated neuroprotection, afforded by increased levels of glutathione peroxidase, is associated with increased expression of PAR-1 in astrocytes in substantia nigra pars compacta [17]; (ii) Transplantation of GDNF-transduced astrocytes to the substantia nigra 1 week prior to an intrastriatal 6-hydroxydopamine lesion provided significant protection of nigral tyrosine hydroxylase-positive cells [12]; (iii) Arundic acid protected dopaminergic neurons against MPTP neurotoxicity in mice and ameliorated neurological deficits, by modulation of astrocytic activation which includes the inhibition of S-100 protein synthesis [18]. It appears plausible that GSTM1 plays a neuroprotective role in astrocytes, and this step may be a critical nurturing element in dopaminergic neuronal survival.

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