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ARTICLE

Exposure to pesticides and heterozygote genotype of GSTP1-Alw26I are associated to Parkinson's disease

Exposição a pesticidas e genótipo heterozigoto de *GSTP1-Alw26I* associam-se à doença de Parkinson

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ABSTRACT

Objective: This study aimed to analyze the frequency of GSTP1-Alw26I polymorphism and to estimate its association with toxic substances in Parkinson's disease (PD). **Methods**: A study group with 154 patients – subdivided into familial and sporadic PD groups – and 158 elderly individuals without the disease (control group) were evaluated. GSTP1-Alw26I polymorphism was analyzed by polymerase chain reaction/restriction fragment length polymorphism (PCR–RFLP). **Results**: Patients were significantly more exposed to pesticides compared with the control group (p=0.0004), and the heterozygote genotype associated to exposure to pesticides also prevailed in patients (p=0.0001). Wild homozygote genotype was related to tobacco use (p=0.043) and alcoholism (p=0.033) in familial PD patients. **Conclusion**: Exposure to pesticides is associated to PD, whose effect can be enhanced when combined with the heterozygote genotype of GSTP1-Alw26I. Also, large genetic and environmental studies considering tobacco use, alcoholism, GSTP1 and PD are necessary to confirm our findings.

Key words: glutathione transferase, genetic polymorphism, Parkinson disease, xenobiotics.

RESUMO

Objetivo: Analisar a frequência do polimorfismo GSTP1-Alw26I, assim como estimar sua associação com substâncias tóxicas na doença de Parkinson (DP). **Métodos:** A casuística avaliada foi composta por um grupo de estudo, com 154 pacientes, subdivididos em DP familial e esporádica, e outro com 158 idosos sem a doença (grupo controle). O polimorfismo GSTP1-Alw26I foi analisado por reação em cadeia da polimerase/polimorfismo de comprimento do fragmento de restrição (PCR/RFLP). **Resultados:** Os pacientes foram significativamente mais expostos a pesticidas, comparados com o grupo controle (p=0,0004), e o genótipo heterozigoto associado a exposição a pesticidas também prevaleceu nos pacientes (p=0,0001). O genótipo homozigoto selvagem apresentou relação com tabagismo (p=0,043) e etilismo (p=0,033) em pacientes com DP familial. Desse modo, a exposição a pesticidas está associada à DP, cujo efeito pode ser potencializado quando combinado ao genótipo heterozigoto de GSTP1-Alw26I. Estudos genético-ambientais envolvendo tabagismo, etilismo, GSTP1 e DP devem ser realizados em casuísticas numerosas, confirmando essa associação.

Palavras-Chave: glutationa transferase, polimorfismo genético, doença de Parkinson, xenobióticos.

Parkinson's disease (PD) is the second most common neurodegenerative disorder, showing high prevalence in elderly patients also in Brazil, with an incidence of 150/200 cases per 100,000 inhabitants[1]. Its pathogenesis includes a complex interaction among genetic and environmental factors[2]. Sporadic cases represent 85% of PD, while 10–15% are familial and less than 5% are monogenic succession, dominant or recessive[3]. Furthermore, polymorphisms have been associated as risk factors for PD[4], including those which determine enzymes involved in xenobiotics metabolism, such as

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glutathione S-transferases (GST) enzymes[5]. Based on biochemistry, immunological and structural proprieties, the GST are divided into eight classes, like π (GSTP) class, whose gene (*GSTP1*) is located in 11q13 human chromosome. Oxidative stress activates GST, and the P1 variant acts on the detoxification of innumerous substances capable of causing acid nucleic, lipid and protein damage[6], especially in the brain[7].

Individual PD risk has been associated to occupational exposure to herbicides and pesticides[8]. There is an association between *GSTP1* genotypes and PD in individuals exposed to pesticides, which shows that the *GSTP1* possibly affects the nigrostriatal response to neurotoxins[7,8]. Also, *GSTP1* polymorphisms can influence onset age for PD[4,9]. On the other hand, there are controversies surrounding the risks or protection, involving *GSTP1*, tobacco use and PD[10]. *GSTP1* polymorphisms have been first described by Board et al.[11] Following studies demonstrated important allelic differences in substrate selectivity, which usually reduces *GSTP1* activity[7,12]. *GSTP1-Alw26I* distribution in population varies among different racial groups, with emphasis on Ile/Ile genotype around 45 and 65%, followed by Ile/Val from 30 to 45% and Val/Val from 5 to 10%, in Caucasian, Chinese and Korean populations[13]. Transitions of nucleotides 313 (AàG), exon 5, and 341 in exon 6 (GàT) were found, involving 2 substitutions of amino acids in the active site of the enzyme (Ile àVal and Val à Ala). The transitions altered the codon 105 of wild enzyme (GSTP1*A) from ATC (Ile) to GTC (Val) in GSTP1*B and GSTP1*C, and modified codon 114 from CGC (Ala) to GTG (Val), in GSTP1*C[7,12]. Associations can be observed between GSTP1*B and sporadic PD[14], especially in patients older than 69 years[4]. Additionally, Shi et al.[15] indicated association of *GSTP1* and PD when studying mice neuronal cells treated with neurotoxic substances. GSTP1*A prevented neuronal loss — contrary to GSTP1*B and GSTP1*C variants[15]. In this context, single nucleotide studies in neurodegenerative diseases can contribute to feature gene-environmental interactions, especially with *GSTP1* and PD, since it affects cellular response to toxicity and interferes in the penetrance of hereditary PD forms and the susceptibility to idiopathic PD. This study aimed to analyze the frequency of *GSTP1-Alw26I* polymorphism in PD, to verify its combination with toxic substances (previous exposure to pesticides, tobacco use and alcoholism) in patients with PD and to estimate its association with the disease onset.

METHODS

Subjects

The studied population consisted of 312 individuals, independently of gender and with mixed racial backgrounds[16]. It was separated into two groups:

- Study group (SG) – n=154 patients; 62.9% men and 36.1% women; average of current age: 68.2±11.6; subdivided into familial PD study group (FSG) – n=33; 69.6% men and 29.4% women; average of current age: 66.1±12, and sporadic PD study group (SSG) – n=121; 61.1% men and 37.9% women; average of current age: 68.8±11.5;
- Control group (CG) – 158 elderly individuals without the disease or familial history of neurodegenerative diseases – 41.1% men and 57.9% women; average of current age: 69.0±8.9.

The FSG was characterized by presenting at least a first or second degree relative with PD diagnosis, and the SSG had no relatives with PD. The patients were seen in Outpatient Neurology Clinic of Hospital de Base of São José do Rio Preto Medical School (FAMERP), Brazil, in the period of 2007 through 2010. They were also subdivided into age groups, in order to provide analysis of the current age — ≤68 and >68 years[4] — and of the disease onset age, therefore defining early PD (EPD), ≤50 years, or late PD (LPD), >50 years[17]. Diagnosis of PD followed the criteria recommended by Jankovic[18], including bradykinesia, rigidity, tremor at rest, postural instability, unilateral onset, response to L-dopa for more than five years, levodopa-induced dyskinesia, progressive disorder, persistent asymmetry and clinical course of ten years or more, as well as complementary tests[18]. The CG belonged to support groups maintained at the same institution. The participants underwent an interview, providing information concerning familial history of chronic-degenerative diseases (PD, Alzheimer's disease, among others) and living habits (previous exposure to pesticides, tobacco use and alcoholism). Exposure to pesticides consisted of any previous occupational exposure to such products, despite its duration[8]. Tobacco use included constant smoking of cigarettes, daily and continuously, for more than six months[10]. Alcoholism included the consumption of at least 40 g of alcohol per day[19]. All subjects were informed of the nature of the study and confirmed their willingness to participate by signing written consent forms. The study was approved by the Ethics Research Committee of the mentioned institution (opinion nº 151/2008 – Certificate of Appreciation Presentation Ethics — CAAE – 0029.0.140.000-08).

Genetic analysis

Analyses of the genetic polymorphism, concerning allele and genotype frequencies for *GSTP1-Alw26I*, were performed in the Laboratory of Molecular Biology of FAMERP. Blood samples were collected and the genomic DNA was extracted from leukocytes by standard procedures[20]. Polymerase chain reaction (PCR) was performed on an Eppendorf Mastercycler (Hamburg, Germany) with 25 μL reaction volumes containing 20 ng of genomic DNA, 1 x PCR buffer (Biosystems, Curitiba, Brazil), 2.5 mM of each primer, 200 μM

of each dNTP, and 1.2 U of Taq DNA polymerase (Taq Gen). The *GSTP1* gene in the DNA sequence was characterized by an A→G transition (Ile105àVal105) at nucleotide 313 (mutation site in exon 5, codon 105), using primers, as previously described[21]. Amplification was performed according to the following protocol: an initial denaturation at 94ºC for 5 minutes followed by 40 cycles of 1 minute at 94ºC and 3 minutes at 62ºC, extension of 90 seconds at 72ºC and a final cycle at 72ºC for 7 minutes. The PCR product was submitted to the *Alw26I* restriction enzyme (Gibco) (5 U per reaction tube) in double boiler at 37ºC, for 16 hours, and separated on 6% polyacrilamide gel for 50 minutes at 180 V. Fragments of 176pb, 91pb and 85pb were identified, and compared with standard Ladder (Invitrogen). One 176pb fragment characterized wild homozygote genotype (I/I), while 176pb, 91pb and 85pb fragments demonstrated heterozygote genotype (I/V); 91pb plus 85pb fragments reveled homozygose V/V (Figure). The DNA fragments were colored by GelRed Nucleic Acid Stain® and visualized by UV illumination.

Statistical analysis

The categorical variables including the allele and genotype frequencies for the *GSTP1* polymorphism were analyzed by means of the Fisher's exact test and the χ^2 test. Statistical analysis also included Hardy–Weinberg equilibrium, *t*-test and binary logistic regression. A level of significance was set at a p-value of 0.05 or less.

RESULTS

The alleles distribution was similar between SG (isoleucine (I)=0.68; valine (V)=0.31), SSG (I=0.66; V=0.33), and CG (I=0.64; V=0.35; p>0.05; Table 1). However, the allele I was significantly higher in familial group (FSG=0.78), compared with CG (0.64; p=0. 036). Wild homozygote (I/I) prevailed in FSG (63.6%) compared with SSG (38.0%; p=0.013). I/V genotype predominated in SG, SSG and CG (50.6, 56.1, and 62.0%, respectively; p>0.05); and V/V, in a reduced frequency, showed similar distribution among groups (p>0.05). FSG exhibited the pattern predicted by Hardy–Weinberg equilibrium (χ^2=0.29; p=0.90), different from SG (χ^2=5.01; p= 0.025), SSG (χ^2=7.82; p=0.005) and CG (χ^2=19.9; p<0.0001).

Table 2 shows socio-demographic data such as age, sex and lifestyle (tobacco use, alcoholism and previous contact with pesticides). Age was similar among groups (p>0.05), whereas male prevailed in SG (62.9%), FSG (69.6%) and SSG (61.1%), compared with CG (41.1%; p=0.002; p=0.005; p=0.001, respectively). Tobacco use and alcoholism had low frequency and were similar among the groups (p>0.05). On the other hand, patients were more exposed to pesticides (50%) than controls (25%; p=0.0004), as well as SSG (53.0%) compared with CG (25%; p=0.0001), meanwhile FSG (38.7%) showed no significant difference from CG (25%; p=0.226).

Tables 3 to 5 present the groups distribution according to genetic variants and environmental factors. SG, SSG and CG were similar concerning tobacco use (Table 3) and alcoholism (Table 4). However, in FSG and smoker patients there

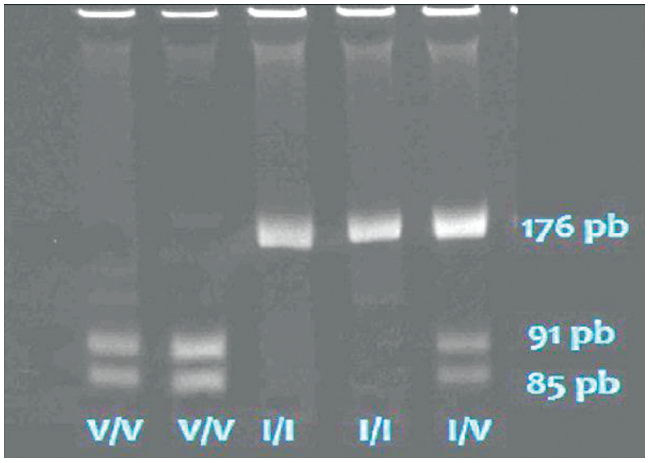

Figure. *GSTP1-Alw 26I* electrophoresis.

Table 1. Genotypic and allelic frequencies for GSTP1-A*lw*26I in patients with Parkinson's disease, grouped in study group, familial or sporadic, and individuals without the disease, or controls.

GSTP1- Alw26I	FSG (a)* n=33		SSG (b)* n=121		SG (c)* n=154		CG (d)* n=158	
Genotype	n	%	n	%	n	%	n	%
I/I	21	63.6	46	38.0	67	43.5	53	33.5
I/V	10	30.3	68	56.1	78	50.6	98	62.0
V/V	2	6.0	7	5.7	9	5.8	7	4.4
Total	33	100	121	100	154	100	158	100
Allele	n	AF	n	AF	n	AF	n	AF
I	52	0.78	160	0.66	212	0.68	204	0.64
V	14	0.21	82	0.33	96	0.31	112	0.35
Total	66	1.00	242	1.00	308	1.00	316	1.00

FSG: familial Parkinson's disease study group; SSG: sporadic Parkinson's disease study group; SG: study group; CG: control group; axd: FSGxCG; bxc: SSGxSG; bxd: SSGxCG; cxd: SGxCG; AF: absolute frequency; p-value: I/I – axd=0.002; axb=0.013; bxd= 0.517; cxd=0.090; I/V – axd=0.001; axb=0.014; bxd=0.390; cxd=0.055; V/V – axd=0.655; axb=1.000; bxd=0.812; cxd=0.757; I/V – axd=0.036; axb=0.068; bxd=0.769; cxd=0.294. χ^2: or Fisher's tests, p<0.05 significance.

Table 2. Social – demographic data in patients with Parkinson's disease, grouped in study group, familial or sporadic, and individuals without the disease, or controls.

	SG (a)* n=154		FSG (b)* n=33		SSG (c)* n=121		CG (d)* n=158	
	n	%	n	%	n	%	n	%
Age (years)								
Median	68.2		66.1		68.8		69.0	
Standart deviation	11.6		12.0		11.5		8.9	
Sex								
Male	97	62.9	23	69.6	74	61.1	65	41.1
Female	57	37.0	10	30.3	47	38.8	93	58.8
Smoking								
Yes	55	38.1	8	25.8	47	41.5	50	37.0
No	89	61.8	23	74.1	66	58.4	85	62.9
Total	144	100	31	100	113	100	135	100
Alcoholism								
Yes	46	31.9	11	35.4	35	30.9	37	27.4
No	98	68.0	20	64.5	78	69.0	98	72.5
Total	144	100	31	100	113	100	135	100
Previous contact with pesticides								
Yes	72	50	12	38.7	60	53.0	21	25.0
No	72	50	19	61.3	53	47.0	63	75.0
Total	144	100	31	100	13	100	84	100

FSG: familial Parkinson's disease study group; SSG: sporadic Parkinson's disease study group; SG: study group; CG: control group; axd: SGxCG; bxc: FSGxSSG; bxd: FSGxCG; cxd: SSGxCG; p-value: Age – axd=0.479; bxc=0.54; bxd=0.196; cxd=0.830; Sex – axd=0.0002; bxc=0.485; bxd=0.005; cxd=0.001; Smoking – axd=0.939; bxc=0.163; bxd=0.330; cxd=0.547; Alcoholism – axd=0.485; bxc=0.795; bxd=0.499; cxd=0.634; Pesticides – axd=0.0004; bxc=0.223; bxd=0.226; cxd=0.0001. χ^2: or Fisher's tests, p<0.05 significance.

Table 3. Distribution of Parkinson's disease patients, grouped in study group, familial or sporadic, and individuals without the disease, smokers and no smokers, according to their genotypes for GSTP1-A*lw*26I.

Genotype	SG (a)				FSG (b)				SSG (c)				CG (d)				p-value			
	T		nT		T		nT		T		GEE nT		T		nT					
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	axd	bxd	cxd	bxc
I/I	24	43.6	40	7.8	6	75	13	56.5	18	38.9	26	39.3	16	32.0	26	30.5	0.950	0.839	0.963	0.676
I/V	29	52.7	42	47.1	2	25	8	34.7	27	57.4	35	53.0	34	68.0	53	62.3	0.950	0.313	0.705	0.297
V/V	2	3.6	7	44.9	0	0	2	8.6	2	4.2	5	7.5	0	0	6	7.0	0.485		0.461	1.000
Total	55	100	89	100	8	100	23	100	47	100	66	100	50	100	85	100				

FSG: familial Parkinson's disease study group; SSG: sporadic Parkinson's disease study group; SG: study group; CG: control group; T: smokers; nT: no smokers; Smokers intra-group analysis (II x -/V) – axd: SGxCG=0.305; bxd: FSGxCG=0.043; cxd: SSGxCG=0.662; bxc: FSGxSSG=0.067.
p-value (χ^2 or Fisher): significance for p<0.05.

Table 4. Distribution of Parkinson's disease patients, grouped in study group, familial or sporadic, and individuals without the disease, alcoholics and no alcoholics, according to their genotypes for GSTP1-A*lw*26I.

Genotype	SG (a)				FSG (b)				SSG (c)				CG (d)				p-value			
	A		nA		A		nA		A		nA		A		nA					
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	axd	bxd	cxd	bxc
I/I	22	47.8	42	42.8	8	72.7	11	55	14	40	30	38.4	12	32.4	30	30.6	0.679	0.454	0.926	0.618
I/V	20	43.4	51	52.4	2	18.1	8	40	18	51.4	44	54.4	25	67.5	62	63.2	0.937	0.721	0.968	0.715
V/V	4	8.6	5	5.1	1	9.0	1	5	3	8.5	4	5.1	0	0	6	6.1	0.103	0.250	0.192	1.000
Total	46	100	98	100	11	100	20	100	35	100	78	100	37	100	98	100				

FSG: familial Parkinson's disease study group; SSG: sporadic Parkinson's disease study group; SG: study group; CG: control group; A: alcoholics; nA: no alcoholics; Alcoholics intra-group analysis (II x -/V) – axd: SGxCG=0.232; bxd: FSGxCG=0.033; cxd: SSGxCG=0.672; bxc: FSGxSSG=0.082. p-value (χ^2 or Fisher): significance for p<0.05.

Table 5. Distribution of Parkinson's disease patients, grouped in study group, familial or sporadic, and individuals without the disease, with or without previous contact with pesticides, according to their genotypes for *GSTP1-Alw26I*.

Genotype	SG (a)				FSG (b)				SSG (c)				CG (d)				p-value			
	P		nP		P		nP		P		nP		P		nP					
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%	axd	bxd	cxd	bxc
I/I	23	31.9	41	56.9	5	41.6	14	73.6	18	30.0	26	49.0	7	33.3	18	28.5	0.643	0.901	0.416	0.393
I/V	43	59.7	28	38.8	6	50	4	21.0	37	61.6	25	47.1	13	61.9	41	65.0	0.0001	0.053	0.0002	1.000
V/V	6	8.3	3	4.1	1	8.3	1	5.2	5	8.3	2	3.77	1	4.7	4	6.3	0.265	1.000	0.242	1.000
Total	72	100	72	100	12	100	19	100	60	100	53	100	21	100	63	100				

FSG: familial Parkinson's disease study group; SSG: sporadic Parkinson's disease study group; SG: study group; CG: control group; P: with previous contact with pesticides; nP: without previous contact with pesticides. Pesticides intra-group analysis (II x -/V) – axd: SGxCG=0.904; bxd: FSGxCG=0.918; cxd: SSGxCG=0.991; bxc: FSGxSSG=0.503. χ^2 or Fisher's exact test: significance for p<0.05.

was a 75% prevalence of wild homozygote genotype (I/I), compared with GC (32%; p=0.043). I/I genotype also prevailed in FSG and alcoholic patients (FSG=72.7%; CG= 27.4%; p=0.033). Heterozygote genotype (I/V) combined with pesticides prevailed in SG (60.5%=43 exposed patients/71 heterozygote patients, *versus* CG=24.0%=13 exposed individuals/54 heterozygote individuals; p= 0.0001). Likewise, I/V presented higher frequency combined with exposure to pesticides in SSG (59.6%=37 exposed patients/62 heterozygote patients); and FSG (60%=6 exposed patients/10 heterozygote patients), compared with GC (24.0%=13 exposed individuals/54 heterozygote individuals; p=0. 0002 and p=0.053, respectively).

Earlier PD or late PD patients presented similar distribution for *GSTP1-Alw26I* genotype (p>0.05), also when combined with tobacco use and alcoholism (p>0.05). On the other hand, heterozygote genotype (I/V) prevailed in patients with earlier PD and previous exposure to pesticides (90%), compared with late PD, also exposed (63.9%), with no significant difference between groups, though (p=0.092). Results were also similar considering genotype distribution between ≤68 and >68 years patients (p>0.05).

The logistic regression analysis for sex, age, tobacco use, alcoholism, exposure to pesticides and *GSTP1-Alw26I* genotypes (Logit Y= -0.397997 +1.031093 sex +1.808364 age -0.562085 smoking -0.130537 alcoholism +1.109846 pesticide -0.654763 genotype) pointed male sex (p=0.0034), age >68 years (p<0.0001) and exposure to pesticides (p=0.001) as risk factors for PD.

DISCUSSION

In this study, with mixed ethnic Brazilian casuistics, the *GSTP1-Alw26I* polymorphism genotypic distribution is similar to that in the general population, considering distinct racial groups[8,13,14]. Heterozygote genotype (I/V) prevailed among familial or sporadic PD patients, and also in the control group. This distribution, although supported by some authors[8], is different from other studies[4,14,22]. The V/V homozygote did not show any association with PD, which was also reported by other authors[8,14]. The presence of the Val105 (V) allelic variant of *GSTP1* is associated with the decrease of the enzyme activity, which would favor dopaminergic neurons degeneration in PD[4,15]. However, in this study, the wild genotype (I/I) presented association with familial PD. Studies involving *GSTP1* polymorphisms and familial PD are scarce[9], making population comparisons difficult.

In this study, the familial PD group exhibited the pattern predicted by Hardy–Weinberg equilibrium, different from the pattern of other groups studied, which is also observed in other case-control studies of different genetic polymorphisms[23,24]. The selection criteria adopted in this study was to form groups of older individuals, since PD mainly affects elderly patients. Furthermore, the disease is associated with occupational exposure to pesticides, which is more common in males. This was confirmed by the logistic regression analysis that demonstrated male sex, age >68 years and exposition to pesticides as risk factors for PD. Therefore, the profiles of patient and control groups do not represent the general population concerning sex and age, influencing the distribution of genotypes. FSG also included younger patients, suggesting better representation of the general population. Additionally, absence of Hardy-Weinberg equilibrium would be expected for a wide group of genetic diseases, considering the gene contribution — although modest — for complex diseases. However, given the numerous candidate gene studies in different cases, genetic markers showing disequilibrium are scarce, which allows investigators to ignore the distribution of genotypes suggesting disequilibrium, therefore ignoring valuable information to identify casual polymorphisms[23].

The polymorphism *GSTP1-Alw26I*, when related to tobacco use and alcoholism, was the same in the SG, SSG and CG groups, as well as in other studies involving smoking and PD[10,22,25]. On the other hand, there are references of tobacco protection in PD, including haplotypes of *GSTP1*[10]. The catalytic efficiency of the *GSTP1* variants differs from that of the wild type, and it varies according to the characteristics of the substrates, manly distinct[26]. This explains the protective effect of the mutant allele regarding diol epoxides found in tobacco products[10], in contrast with its reduced effects upon detoxification of pesticides[7]. In this study, there was an association between smoking and wild homozygote

genotype in familial PD patients, confirming the studies by Pal et al.[26] and De Palma et al.[10].

Likewise, association between I/I genotype and alcoholism was found in familial PD patients. Admittedly, alcohol abuse damages brain structures and their functions, which leads to neurodegeneration[27]. The effects of alcohol in the brain are not uniform, affecting mainly the pre- frontal cortex, the hippocampus, the cerebellum, the *substantia nigra* and the glia[27]. Dopaminergic neurons in the substantia nigra are believed to be damaged by alcohol during intrauterine development[27,28]. The effect of alcohol in this region is still unclear concerning brains are already developed[28]. Studies involving GSTs, alcohol, *substantia nigra* and PD are rare. On the other hand, GST activity is known to be reduced in hepatocytes due to alcohol exposure[29]. Retinoid X receptor α-deficient (RXRα KO) mice, which are more susceptible to ethanol-induced hepatotoxicity, showed a 56% decrease in *GSTP1* activity, which demonstrates its role in the detoxification of alcohol in the liver[29]. Therefore, *GSTP1* polymorphisms seem to intensify the damage caused by ethanol in hepatocytes, leading to alcoholic cirrhosis and pancreatitis[19]. The present study revealed the combination of the wild genotype (I/I) with alcoholism in familial PD patients. The small casuistic of familial patients analyzed in this study speaks in favor of detailed studies involving familial PD patients, *GSTP1* polymorphisms and lifestyle.

PD patients group showed more exposure to pesticides, especially concerning the combination of such exposure and the heterozygote genotype (I/V). The GST enzymes, mainly *GSTP1*[4,7], are involved in metabolizing pesticides[8]. Studies demonstrated that some kinds of pesticides, like rotenone, can cause PD-like symptoms[30], acting as inhibitors of mitochondrial I complex. In this case, there is some evidence that dopaminergic neurons are particularly vulnerable to mitochondrial dysfunction[30]. The toxin is captured by dopamine and noradrenalin transporters and is accumulated in the cytosol, causing cellular death induced by oxidative stress and deficiency of the breathing mitochondrial chain[30]. Shi et al.[15] demonstrated protection of the neuronal dopaminergic cells of mice exposed to rotenone by the expression of the wild *GSTP1* (I allele), and reduced protection by its variants expression (V allele). These data were confirmed in the present study, by means of the association of *GSTP1-Alw26I* heterozygote, exposure to pesticides and PD.

The V allele have been associated to late onset PD, given its prevalence in patients with more than 68 years of age[4,15], despite the lack of association between *GSTP1* polymorphisms and PD onset age in North American casuistics[14]. In this study, there was no association of *GSTP1-Alw26I* genotypes, current age and PD onset age. However, considering onset age, previous exposure to pesticides and the presence of the mutant allele of *GSTP1-Alw26I*, the group with earlier PD (PD onset before than 50 years) was significant, if compared with those with late PD onset age, which requires confirmation in wide casuistics.

The regulation of the apoptotic kinase c–jun terminal (JNK) by protein–protein direct binding and the detoxification of electrophilic compounds by conjugation with reduced glutathione have been the mainly reported *GSTP1* functions[15]. The former mechanism could be associated to late onset PD in *GSTP1* heterozygote patients. The *GSTP1* enzyme would play an important role in the thermal shock protein system — also responsible for regulating JNK apoptosis[4] failed to act due to aging. Shi et al.[15] demonstrated that in the brains of mice treated with rotenone there was no activation of the JNK pathway of apoptosis. High level of oxidative stress was detected though, and *GSTP1* detoxification with reduced glutathione was essential for neuron protection in this case[15]. That would explain the possible relation suggested in this study, between heterozygote genotype for *GSTP1* in patients with previous exposure to pesticides, and the earlier PD, to be confirmed in further studies.

In conclusion, this study confirms the association among PD, male sex, ageing and exposure to pesticides, whose effects can be enhanced in combination with I/V genotype of *GSTP1-Alw26I,* reinforcing the relation between genetic polymorphisms involved in xenobiotics metabolism and environmental factors in PD. This is confirmed by the prevalence of the I/I genotype and tobacco or alcohol use only in the FSG, suggesting that different effects of *GSTP1-Alw26I* variants, due to lifestyle, determine specific subgroups of patients, which may be confirmed in other casuistics.

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