The COMT *L* allele Modifies the Association between MAOB Polymorphism and PD in Taiwanese

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Article abstract

Objective: Reports suggest that COMT$^{L/L}$ (Val^{158}/Met) and MAOB intron 13 genotype polymorphism is associated with Parkinson's disease (PD). To understand the ethnicity-specific effects of genetic polymorphism, we performed a case-control study of the association between PD susceptibility and polymorphism of MAOB and COMT, both separately and in combination, in Taiwanese. *Methods:* 224 PD patients and 197 controls, matched for age, gender, and birthplace, were recruited. MAOB and COMT polymorphism genotyping was performed using PCR-based RFLP analyses. Chi-square, odds ratio, and Fisher's exact tests were used to compare differences in allelic frequencies and genotypes. *Results:* The MAOB *G* genotype (*G* in men and *G/G* in women) was associated with a 2.07-fold increased relative risk of PD. COMT polymorphism, considered alone, showed no correlation with PD risk; however, a significant synergistic enhancement was found in PD patients harboring both the COMT^{L} and MAOB *G* genotypes. *Conclusions:* These results suggest that, in Taiwanese, PD risk is associated with MAOB *G* intron 13 polymorphism and this association is augmented in the presence of the COMT^{L} genotype, indicating an interaction of these two dopamine metabolizing enzymes in the pathogenesis of sporadic PD. However, the relatively low frequencies of these combined genotypes in our study necessitates confirmation with a larger sample size.

Introduction

Since PD is characterized by the loss of at least 60% of midbrain dopaminergic neurons[1], it has been suggested that genetic variants of the enzymes involved in the biosynthesis and degradation of dopamine and related compounds influence susceptibility to development of this disease. These enzymes include monoamine oxidase B (MAOB) and catechol-*O*-methyltransferase (COMT). MAOB is potentially relevant to PD because of its role in catabolizing dopamine, with the resultant production of reactive oxidative free radicals,[2,3] and in activating exogenous neurotoxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin that induces parkinsonism in intravenous users of synthetic heroin and in nonhuman primates.[4-6] Inhibition of MAOB activity by L-deprenyl prevents the development of parkinsonism in nonhuman primates by blocking the conversion of MPTP to MPP^+,[7] and possibly reduces the rate of PD progression in humans.[8] PD patients are reported to have higher platelet MAOB activity than control individuals.[9] Furthermore, inhibition of MAOB activity in the brains of cigarette smokers has been suggested to have a protective effect against the development of PD.[10,11] Thus, there is an increasing body of evidence that MAOB may play a crucial role in the pathogenesis of PD.

The gene encoding MAOB is located on the X chromosome. It contains a single-stranded conformational polymorphism in intron 13, a transitional conversion of adenine (*A*) to guanine (*G*) at a position 36 base pairs (bp) upstream from the 5' end of exon 14.[12] Although several

studies have been carried out on a possible association between the *G-A* polymorphism and PD, the findings have been inconsistent. Both the *A*[12] and *G*[13] alleles have been reported to be associated with a risk of PD in Caucasians, while two studies failed to find any correlation in Caucasian populations.[14,15] In Asian populations, no correlation was found between PD occurrence and MAOB *G-A* polymorphism in either Japanese[16] or a small Taiwanese population.[17]

COMT polymorphism has also been studied in terms of an association with PD risk. COMT is a ubiquitous enzyme that catalyzes the *O*-methylation of biologically active or toxic catechols, and plays a major role in the metabolism of drugs and neurotransmitters, such as L-dopa, dopamine, noradrenaline, and adrenaline. A single gene, located on chromosome 22q11, encodes both the acid-soluble and membrane-bound forms of this enzyme. A *G* to *A* transition at codon 158 of the COMT gene, resulting in the substitution of methionine for valine, is linked to low COMT enzyme activity and is designated the *L* (low activity) allele, in contrast to the *H* (high activity) allele.[18] In individuals with the *L* allele, the COMT protein is thermolabile. Differences in COMT activity may determine individual variations in the therapeutic response to levodopa[19] and affect the individual's susceptibility to PD. Studies suggest that COMT^{L} is less frequent in Asians than in Caucasians.[20,21] Homozygosity for the COMT *L* allele has been reported to be a genetic risk for PD in Japanese[20], but recent studies in Caucasians and Chinese failed to find any association between PD and COMT polymorphism.[22,23]

The prevalence of PD varies world-wide. In general, this disease is less common in Asia than in Western countries.[24] Thus, understanding the ethnicity-specific effects of susceptibility genes on PD risk in different races may provide valuable clues to potential causes of racial and individual susceptibility to PD. Although several studies have investigated a possible association of either MAOB or COMT polymorphism with PD susceptibility in Asian populations,[16,17, 20,23,25] the results have been inconsistent, possibly due to differences in ethnicity, sample size, and the matched controls used. Moreover, all previous studies have focused on a single gene effect of either MAOB or COMT. In the present study, we determined both the individual and combined effects of MAOB and COMT genetic polymorphisms on PD risk in a large population of Taiwanese PD patients (n=224) and in age-, sex-, and origin (birthplace)-matched control subjects (n=197).

Materials and Methods

Human subjects

After obtaining their informed consent, 224 patients with idiopathic PD (162 men and 62 women; average age: 67.2 ± 9.1 years) were recruited at the Movement Disorder Clinics of the National Taiwan University Hospital, Taipei and the Taipei Veterans General Hospital. All patients met the criteria for PD, which included the presence of two of the three cardinal signs (tremor at rest, bradykinesia, and rigidity), improvement of symptoms with L-dopa therapy, and no evidence of secondary parkinsonism caused by other neurological diseases or known drugs

or toxins, or of atypical parkinsonism. The control group consisted of 197 individuals (145 men and 52 women; average age: 65.8 ± 9.2 years) recruited from the same two hospitals and from community groups in the Taipei area, chosen on the basis that neither they nor their blood relatives showed evidence of any neurological and psychiatric disorders. Controls and cases were matched for age, sex, and birthplace (Table 1).

Laboratory analysis

(i) DNA extraction

Samples of buccal mucosa cells were collected from each PD patient or control subject by rolling a buccal brush along the inner surface of the cheek. The brush was dried for 10-15 minutes at room temperature, then immediately stored at 4ºC until extraction of the genomic DNA, performed using a QIAamp DNA Mini kit (QIAGEN Inc., Chatsworth, CA, U.S.A.). The purified DNA was stored at -20ºC for genotype analysis.

(ii) Identification of MAOB intron 13 and COMT polymorphisms

PCR-based RFLP analyses were used to determine the MAOB and COMT genotypes of the subjects.

In the MAOB study, a 232 bp DNA fragment of the MAOB gene containing the intron 13 polymorphism was amplified. The forward primer 5'-GGAACCTCTTATACCACAGG-3' and reverse primer 5'-GACTGCCAGATTTCATCCTC-3' were used for partial MAOB DNA fragment amplification.[12] The PCR reaction was carried out in a final volume of 50 μL containing 5 μL of 10X Taq buffer, 2 μL of 0.25 mM dNTP mix, 20 pmol of each primer, 100

ng of genomic DNA as a template, 3 mM $MgCl_2$, and 1 unit of Ampli Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT). The PCR procedures involved an initial denaturation at 94°C for 4 min, 34 cycles of 94°C for 40 sec, 54°C for 30 sec, and 72°C for 40 sec, and a final extension at 72°C for 10 min. To determine the MAOB polymorphism, 10 μL aliquots of the PCR-amplified DNA product were digested with restriction enzyme Tsp45I and the digested DNA pattern analyzed by electrophoresis on a 2% SeaKem LE agarose gel (FMC Crop., Rockland, ME). MAOB allele 1 (containing A and therefore the Tsp45I restriction site) was detected as two bands of 146 and 86 bp, while allele 2 (containing G, no Tsp45I restriction site) was detected as a single 232 bp band (Figure 1).

In the COMT study, the PCR mixture contained the same reagents used in MAOB amplification, except that the forward primer was 5'-TCGTGGACGCCGTGATTCAGG-3' and the reverse primer 5'-AGGTCTGACAACGGGTCAGGC-3'.[25] The reaction mixture was subjected to an initial denaturation at 94ºC for 4 min, followed by 34 cycles of 94ºC for 40 sec, 55ºC for 30 sec, and 72ºC for 30 sec, and a final extension at 72ºC for 10 min. Ten microliters of the amplified double-stranded DNA was digested with restriction endonuclease *Nla*III, then the sample was subjected to electrophoresis. The $COMT^H$ allele was detected by the presence of two bands of 136 and 81 bp and the $COMT^L$ allele by the presence of three bands of 96, 81, and 40 bp (Figure 1). To ensure the validity of the data, the experiments were repeated.

Statistical analysis

In most cases, chi-square and odds ratio tests were used to compare either allelic frequencies or the genotype frequencies of MAOB or COMT in PD patients and control subjects. When more than 20% of the cell numbers that the expected number of cases was less than 5.0 in a cell, or when the expected number of cases was less than 1.0 in a cell, Fisher's exact test was performed.[26] Since the MAOB gene is located on the X chromosome, results for the MAOB genotype were assessed separately in men and women. To comprehensively assess the individual contributions of MAOB and COMT polymorphism to an association with PD risk, we further examined the relationship of the individual susceptibility genotypes (the MAOB *G* allele and the COMT *L* allele) with PD risk, stratified by age (≤ 60-years-old vs. >60-years-old) or birthplace (Taiwan vs. mainland China).

Because of the possibility of a potential interaction of the two dopamine-metabolizing genes in the development of PD, we then investigated PD risk associated with combinations of these two susceptibility genotypes. All statistical tests were based on a two-tailed probability and values of $p<0.05$ were considered significant.

Results

MAOB G-A polymorphism in intron 13

MAOB genotypic data were available for 220 patients (98.2%) and 191 controls (97.0%).

Table 2 shows the allelic frequencies and genotype distribution for MAOB polymorphism in PD patients and controls. There was no significant difference in total *A* and *G* MAOB allelic frequencies between PD patients (81.6% and 18.4%, A:G ratio = 4.42) and controls (86.4% and 13.6%, A:G ratio = 6.33). However, MAOB genotype distribution showed a significant difference between the two groups, the frequency of the *G* genotype (*G* in men and *G/G* in women) being twice as high in PD patients compared to controls (OR = 2.07, 95% CI 1.12 to 3.81, $p = 0.018$ for χ^2 test). This association showed a significant gender difference. In men, the association between the MAOB *G* genotype and an increased PD risk was slightly stronger (OR = 2.14, 95% CI 1.14 to 4.00, $p=0.016$ for χ^2 test) than in all subjects combined, and a significantly reduced risk of PD was found in subjects with the *A* allele (OR = 0.47, 95% CI 0.25 to 0.88), while, in women, there was no significant difference in the distribution of MAOB genotypes between PD patients and controls. Compared with previous data for a Caucasian population[13], the frequency of the homozygous variant *G* in women was much lower in Taiwanese (17.2% vs.1.6%).

Table 3 shows the results of analyses of the effects of age and birthplace on the association of the susceptible MAOB *G* genotype with PD risk. A higher frequency of the MAOB *G* genotype was seen in PD patients in both age groups (*p* values of 0.056 for subjects ≤ 60 years and of 0.053 for subjects >60 years). Interestingly, the frequency of the *G* genotype was higher in subjects born in mainland China compared with those born in Taiwan; in the mainland

Chinese group, the frequency was 25.2% in PD patients, 13.8% in controls, and 20.0% in all subjects combined, while, in patients born in Taiwan, the corresponding frequencies were 9.6%, 4.8%, and 7.3%, a 2.6- to 2.9-fold difference. Statistically, an association between MAOB genotype and PD risk was seen in subjects born in mainland China ($\chi^2 = 3.86$, p=0.049), but not in those born in Taiwan ($\chi^2 = 1.87$, $p = 0.171$).

COMT polymorphism at codon 158

COMT genotype data were available for 222 patients (99.1%) and 191 controls (97.0 %). Table 4 shows the allelic and genotypic frequencies for COMT polymorphism in PD patients and controls. The allelic frequency of COMT^L (Met) was 25.9% in PD patients, and 22.5% in control subjects, this difference not being significantly different. There was also no significant difference between the two groups in the frequencies of either the homozygous (*H/H* or *L/L)* or heterozygous (*H/L*) genotypes. Subgroup analyses for gender, age, and birthplace also showed no association between the susceptible COMT genotype (COMT^L) and PD risk (Table 5).

Combined effect of the two susceptibility genes

Combined MAOB and COMT genotyping data were available for 218 PD patients (97.3%) and 185 controls (94.0%). The distribution of the joint allele frequencies for these two loci is shown in Table 6. Importantly, the frequency of the combined alleles MAOB *A* and COMT *H* was lower in PD patients than in controls (OR 0.76, 95% CI 0.59-0.98, $p = 0.033$), whereas the

frequency of the combined alleles MAOB *G* and COMT *L* was 2.4 times higher in PD patients than in controls (OR = 2.39, 95% CI 1.11-5.18, $p = 0.023$). Moreover, these associations between PD and the combined MAOB and COMT alleles were stronger in men than in the total population, but were not seen in women. In men, the combined alleles MAOB *A* and COMT *H* were associated with a reduced relative risk of PD (OR 0.65, 95% CI 0.46-0.91, $p = 0.013$), whereas the combined alleles MAOB *G* and COMT *L* markedly increased the relative risk of PD by 7.24-fold (95% CI 1.65-31.80, $p = 0.002$). These findings suggest that the combination of the MAOB *A* and COMT *H* alleles may be a protective factor for PD development, while the combination of the uncommon MAOB *G* and COMT *L* alleles may increase the relative risk of PD.

Table 7 shows the combined effect of these two susceptibility genotypes on the development of PD. For MAOB polymorphism, the *AA* and *A/G* genotypes were combined because the two genotypes were found to have a similar effect, relative to *GG* (Table 2). For COMT polymorphism, because no association of the genotype with PD was found in this study (Table 4), the heterozygous genotype (*H/L*) was combined with either the homozygous *HH* or *LL* genotypes**.** Consistently, the presence of the MAOB *G* genotype (G for men and G/G for women) and of at least one copy of the COMT *L* allele significantly increased the relative risk of PD by a factor of 5.0-6.0, suggesting a strong synergistic effect of the low activity COMT *L* allele on the development of PD in subjects with the MAOB *G* gene. Interestingly, the

frequency of the combined genotypes of MAOB *A, A/A, and A/G* with COMT *H/H and H/L* was lower in PD patients than in controls ($p = 0.02$), implying a protective effect of the combined wild and heterozygous genotypes of these two dopamine metabolizing enzymes on the occurrence of PD (OR = 0.55).

Discussion

The frequency of the MAOB *G-A* polymorphism in intron 13 differs in the ethnic groups that have been studied. In Caucasians, the frequency of intron 13 allele 1 (*A*) of the MAOB gene is reported to be 45-51% in PD patients and 49-58% in controls.[12-15] Consistent with other studies in Asians,[16,17] our present results showed a predominant distribution of the *A* allele (*A/G* ratio = 4.43) in control subjects, and no association between PD and MAOB polymorphism in terms of the total *A* and *G* allele frequencies. However, when we assessed the gender distribution of the MAOB genotypes, we found an increased frequency of the MAOB *G* variant both in men and in the whole group, a finding not previously reported in studies on Asians. Moreover, an inverse relationship between PD and *A* polymorphism was seen in men, with a reduced relative risk of 0.47(95% CI 0.25,0.88). The sample size in the current study was substantially greater than those in previous studies involving Japanese[16] and Taiwanese,[17] thus providing a greater power to detect a significant difference in the distribution of genotype frequencies between PD and control subjects. The prevalence rate of PD is 2- to 4-fold higher in Caucasians than in Asians.[24] If the *G* variant of MAOB indeed increases the risk of PD

development, a lower frequency of this genotype would be expected in Asians than in Caucasians. Moreover, the MAOB gene is on the X-chromosome, suggesting that any effect of MAOB on PD risk should be higher in men than in women. Importantly, our findings are consistent with both of these propositions. Although the *G-A* polymorphism occurs in an intron and, therefore, does not directly alter the amino acid sequence of the enzyme, linkage disequilibrium of this polymorphism with other genes that confer PD susceptibility might explain this association.[13,27] PD patients are reported to have higher MAOB activity than controls.[9] The positive association between the MAOB *G* polymorphism and PD seen in this study justifies further investigations to determine whether this polymorphism affects brain MAOB activity, since high MAOB activity may increase oxidative stress and PD risk.[3]

The Taiwanese population is ethnically diverse. The majority of the population is Han Chinese and the rest aboriginal. The Taiwan aborigines consist of at least 11-13 linguistically distinct populations and those groups settled in the plains or valleys are known collectively as Peen-Poof.[28] Several centuries ago, the Han Chinese began to migrate from mainland China to Taiwan. In the early migration periods, they settled in the south of Taiwan. Most were single men who married Peen-Poof women, resulting in an ethnic admixture.[29] This migration steadily continued for several centuries. However, a recent large migration of Han Chinese to Taiwan occurred approximately 50 years ago after the Second World War; this group of Chinese population probably differs ethnically from the descendents of marriages between Peen-Poof

and previous Han immigrants. Interestingly, we found a significant difference in the frequency distribution of MAOB polymorphism and its association with PD in the two subgroups of populations born in mainland China or Taiwan. This finding is compatible with the ethnic diversity of Taiwanese resulting from the history of migration. Thus, any genetic association study of PD in Taiwanese should be conducted using patient and control populations in which ethnicity is closely matched.

The level of COMT enzyme activity is genetically polymorphic. Some ethnic differences have been recognized and may explain variations in the individual response to L-dopa therapy.[19] The low COMT activity allele ($COMT^{L}$) is common in Caucasians, with an allelic frequency of 40-50%[21,22], but less common in Asians in whom the frequency is 20-30%.[20,21,23,25,30] In agreement with the results of another population study in Taiwan,[30] we found a low $COMT^{L}$ allele frequency in our subjects; in controls, the frequency of the *L* allele was 22.5% and the frequency of the *L/L* homozygote 6.3%. As in a previous study in Hong-Kong Chinese,[23], no correlation was found in the present study between COMT 158Met/Val polymorphism and PD.

In this study, the most noteworthy finding was that individuals harboring both the MAOB *G* genotype (*G* for men and *G/G* for women) and the $COMT^{L}$ allele had a much higher risk of PD than those harboring only the MAOB *G* genotype. This synergistic effect of the low activity $COMT^{L}$ allele on PD risk in individuals harboring MAOB *G* suggests that an interaction of

these two enzyme polymorphisms may be one of the underlying mechanisms of PD etiology. In humans, both COMT and MAOB metabolize dopamine and other catecholamines. Low COMT enzyme activity results in dopamine being metabolized mainly by MAO, which may increase oxidative stress on midbrain dopamine neurons as a result of free radical formation[3,31] and thus trigger or enhance the development of PD in susceptible individuals. Accordingly, the MAOB *G* genotype may be a major susceptible risk factor and COMT^{L} may facilitate the development of PD in Taiwanese. However, because of the relatively low frequencies of the combined MAOB *G* and COMT *L* alleles in this study, further confirmation with a larger sample size is necessary.

A full understanding of the pathogenesis of PD requires extensive investigation to evaluate whether a cluster of related genes is involved in the nigrostriatal degeneration of dopamine neurons. To date, most studies have focused on single genetic risk susceptibility to PD. However, such isolated single gene studies may overlook the complexity of dopamine metabolism, which involves sequential biochemical processes, and the effect of multiple genes on PD development. As far as we are aware, our study is the first to address the issue of dopamine catabolism in relation to PD risk using a two-gene model. This approach should allow more precise evaluation of PD risk associated with MAOB and COMT polymorphisms and could also be used to assess associations between PD and other candidate genes, such as CYP 2D6, CYP 1A1, and CYP 2E1, that potentially contribute to dopaminergic cell death.[1]

Tables

Table 1 *Demographic data for PD patients and control subjects*

	PD patients N (%)	Control subjects N (%)	*p* Value[a]
Total	224	197	
By gender:			0.768
Men	162 (72.3)	145 (73.6)	
Women	62 (27.7)	52 (26.4)	
By age:			0.839
≤ 60 years	37 (16.52)	34 (17.26)	
>60 years	187 (83.48)	163 (82.74)	
By birthplace:			0.500
Taiwan	115 (52.0)[b]	109 (55.3)	
Mainland China	106 (48.0)	88 (44.7)	

[a] χ^2 test.

[b] The place of birth of 3 PD patients was uncertain.

Table 2 *Distribution of MAOB allele frequency and genotype polymorphism, and estimated OR in relation to PD risk*

	PD patients (n=220) No. (%)	Control subjects (n=191) No. (%)	χ^2	*p* Value	OR (95% CI)
Total alleles			2.211	0.137	
A	230 (81.6)	209 (86.4)			0.698 (0.434, 1.123)
G	52 (18.4)	33 (13.6)			1.432 (0.891, 2.302)
*Genotype			6.042	0.049*	
A, A/A	169 (76.8)[a]	158 (82.7)	2.192	0.139	0.692 (0.425, 1.128)
A/G	14 (6.4)[b]	16 (8.4)	0.612	0.434	0.743 (0.353, 1.566)
**G,G/G*	37 (16.8)[c]	17 (8.9)	5.616	0.018*	2.069 (1.124, 3.811)
*Men			5.749	0.016*	
**A*	122 (77.2)	123 (87.9)			0.468 (0.250, 0.878)
**G*	36 (22.8)	17 (12.1)			2.135 (1.138, 4.004)
Women			N.A.	0.392^	
A/A	47 (75.8)	35 (68.6)	0.724	0.395	1.432 (0.625, 3.282)
A/G	14 (22.6)	16 (31.4)	1.109	0.293	0.638 (0.276, 1.477)
G/G	1 (1.6)	0 (0.0)	N.A.	1.00^	N.A.

* : Significant difference between the two groups.

^ : Fisher's exact test.

N.A. : not applicable.

The genotype frequency in women conformed to the Hardy-Weinberg equilibrium.

Table 3 *Odds ratio for MAOB genotype polymorphism in relation to PD risk stratified by age or birthplace*

	PD patients (n=220) No. (%)	Control subjects (n=191) No. (%)	χ^2	*p* Value	OR (95% CI)
Age					
≤ 60 years			N.A.	0.056^	
[a]*A*	33 (86.8)	34 (100)			N.A.
[b]*G*	5 (13.2)	0 (0)			N.A.
>60 years			N.A.	0.053^	
A	150 (82.4)	140 (89.2)			0.569 (0.303, 1.071)
G	32 (17.6)	17 (10.8)			1.757 (0.934, 3.304)
[c]Birthplace					
Taiwan			1.874	0.171	
A	103 (90.4)	99 (95.2)			0.473 (0.159, 1.410)
G	11 (9.6)	5 (4.8)			2.115 (0.709, 6.305)
*Mainland China			3.864	0.049*	
A	77 (74.8)	75 (86.2)			0.474 (0.223, 1.007)
G	26 (25.2)	12 (13.8)			2.116 (0.993, 4.487)

* : Significant difference between groups. ^ : Fisher's exact test. N.A. : not applicable.

[a]*A* corresponds to genotypes *A/A* and *A/G*

[b]*G* corresponds to hemizygous *G* (men) and homozygous *G* (women)

[c] The place of birth of 3 PD patients was uncertain.

Table 4 *COMT allele and genotype frequencies in PD patients and control subjects*

	PD patients (n=222) No. (%)	Control subjects (n=191) No. (%)	χ^2	*p* Value	OR (95% CI)
Total alleles			1.280	0.258	
H(Val)	329 (74.1)	296 (77.5)			0.831 (0.603, 1.145)
L(Met)	115 (25.9)	86 (22.5)			1.203 (0.873, 1.658)
Genotype			1.194	0.550	
H/H	125 (56.3)	117 (61.2)	1.037	0.309	0.815 (0.550, 1.208)
H/L	79 (35.6)	62 (32.5)	0.446	0.504	1.149 (0.764, 1.730)
L/L	18 (8.1)	12 (6.3)	0.508	0.476	1.316 (0.617, 2.807)

The genotype frequency in each group was within the Hardy-Weinberg equilibrium.

Table 5 *Odds ratio for COMT genotype polymorphism in relation to PD risk stratified by age or birthplace*

	PD patients (n=222) No. (%)	Control subjects (n=191) No. (%)	χ^2	*p* Value	OR (95% CI)
Gender			1.689	0.639	
Men			1.488	0.223	
H/H	93 (58.1)	91 (65.0)			0.747 (0.468, 1.194)
H/L, L/L	67 (41.9)	49 (35.0)			1.338 (0.838, 2.137)
Women			0.122	0.727	
H/H	32 (51.6)	28 (54.9)			0.876 (0.417, 1.842)
H/L, L/L	30 (48.4)	23 (45.1)			1.141 (0.543, 2.400)
Age			4.059	0.255	
≤ 60 years			0.589	0.443	
H/H	25 (61.0)	33 (68.7)			0.710 (0.296, 1.704)
H/L, L/L	16 (39.0)	15 (31.3)			1.408 (0.587, 3.379)
>60 years			0.782	0.377	
H/H	100 (55.2)	86 (60.1)			0.818 (0.524, 1.277)
H/L, L/L	81 (44.8)	57 (39.9)			1.222 (0.783, 1.907)
[a]Birthplace			3.070	0.381	
Taiwan			0.320	0.571	
H/H	66 (57.4)	63 (61.2)			0.855 (0.497, 1.470)
H/L, L/L	49 (42.6)	40 (38.8)			1.169 (0.680, 2.010)
Mainland China			2.673	0.102	
H/H	54 (51.9)	56 (63.6)			0.617 (0.345, 1.102)
H/L, L/L	50 (48.1)	32 (36.4)			1.620 (0.907, 2.895)

[a] The place of birth of 3 PD patients was uncertain.

Table 6 *Distribution of the combined allele frequencies of MAOB and COMT, and estimated OR in relation to PD risk*

			PD patients (n=218)	Controls (n=185)			
	MAOB	COMT	No. (%)	No. (%)	χ^2	*P* Value	OR (95% C.I.)
Total					7.899	0.048*	
	A	*H*	322 (59.29)	309 (65.74)	4.536	0.033*	0.759 (0.588-0.978)
	A	*L*	128 (22.86)	97 (20.64)	0.737	0.391	1.139 (0.846-1.535)
	G	*H*	75 (13.39)	55 (11.70)	0.662	0.416	1.167 (0.805-1.692)
	G	*L*	25 (4.46)	9 (1.91)	5.203	0.023*	2.394 (1.106-5.180)
Men					14.503	0.002*	
	A	*H*	179 (57.37)	184 (67.41)	6.189	0.013*	0.651 (0.464-0.914)
	A	*L*	65 (20.83)	56 (20.74)	0.001	0.978	1.006 (0.673-1.502)
	G	*H*	52 (16.67)	30 (11.11)	3.691	0.055	1.600 (0.988-2.592)
	G	*L*	16 (5.13)	2 (0.74)	9.296	0.002*	7.243 (1.650-31.795)
Women					2.285	0.515	
	A	*H*	153 (61.69)	127 (63.50)	0.154	0.695	0.926 (0.630-1.361)
	A	*L*	63 (25.40)	41 (20.50)	1.493	0.222	1.321 (0.645-2.064)
	G	*H*	23 (9.27)	25 (12.50)	1.204	0.272	0.716 (0.393-1.304)
	G	*L*	9 (3.63)	7 (3.50)	0.005	0.942	1.038 (0.380-2.839)

* : Significant difference between groups

Table 7 *Estimated odds ratio for PD development associated with the coexistence of both MAOB and COMT susceptibility genotypes*

		PD patients (n=218)	Controls (n=185)			
MAOB	COMT	No. (%)	No.(%)	χ^2	Value	OR (95% CI)
Combined genotypes				9.845	0.080	
A, A/A, A/G	*H/H*	99 (45.41)	100 (54.05)	2.990	0.084	0.707(0.477-1.048)
A, A/A, A/G	*H/L*	67 (30.73)	58 (31.35)	0.018	0.894	0.972 (0.636-1.484
A, A/A, A/G	*L/L*	17 (7.80)	11 (5.95)	0.531	0.466	1.338 (0.610-2.933)
G, G/G	*H/H,*	21 (9.63)	14 (7.57)	0.538	0.463	1.302 (0.642-2.639)
G, G/G	*H/L*	12 (5.50)	2 (1.08)	5.840	0.016*	5.330 (1.177-24.131)
G, G/G	*L/L*	2 (0.92)	0 (0.00)	N.A.	0.502^	N.A.
Combined genotypes				6.724	0.081	
A, A/A, A/G	*H/H, H/L*	166 (76.15)	158 (85.41)	5.443	0.020*	0.546 (0.326-0.912)
A, A/A, A/G	*L/L*	17 (7.80)	11 (5.95)	0.531	0.466	1.338 (0.610-2.933)
G, G/G	*H/H, H/L*	33 (15.14)	16 (8.65)	3.945	0.047*	1.884 (1.001-3.546)
G, G/G	*L/L*	2 (0.92)	0 (0.00)	N.A	0.502^	N.A
Combined genotypes				9.235	0.026*	
A, A/A, A/G	*H/H*	99 (45.41)	100 (54.05)	2.990	0.084	0.707 (0.477-1.048)
A, A/A, A/G	*H/L,L/L*	84 (38.53)	69 (37.30)	0.065	0.799	1.054 (0.704-1.578
G, G/G	*H/H,*	21 (9.63)	14 (7.57)	0.538	0.463	1.302 (0.642-2.639)
G, G/G	*H/L,L/L*	14 (6.42)	2 (1.08)	7.088	0.006*	6.279 (1.408-28.001)

*: Significant difference between groups

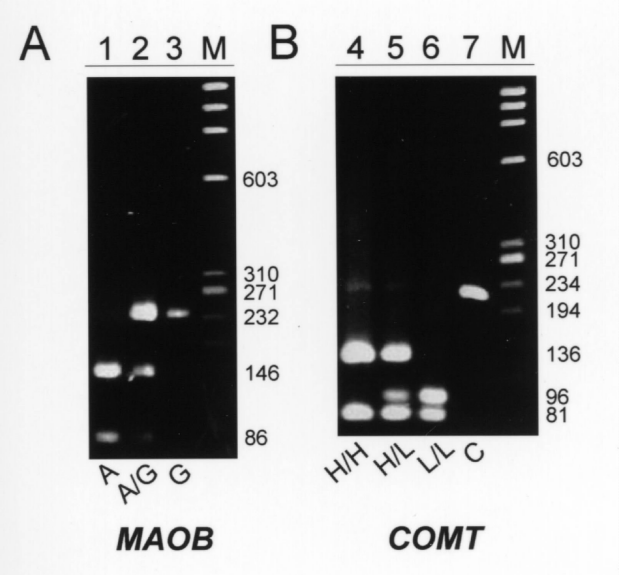
^ : Fisher's exact test

N.A.: not applicable

Figure Legend

Figure 1. PCR-based RFLP analysis of genetic polymorphisms of MAOB (A) and COMT (B). M : molecular weight marker. Lanes 1 to 3 show MAOB intron 13 polymorphisms : lane 1, the A allele; lane 2, the A/G heterozygote; and lane 3, the G allele. Lanes 4 to 6 show COMT polymorphisms : lane 4; the wild-type allele (high enzyme activity); lane 5, the heterozygote; and lane 6, the homozygous variant allele (low enzyme activity). Lane 7 shows the PCR-amplified COMT product.

Figure 1.


A
1 2 3 M
603
310
271
232
146
86
A
A/G
G
MAOB
B
4 5 6 7 M
603
310
271
234
194
136
96
81
H/H
H/L
L/L
C
COMT

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