An association study between genetic variants at mu-opioid receptor, dopamine transporter, catechol-O-methyltransferase, and dopamine genes and risk of Parkinson's disease

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Abstract

Background: Parkinson's disease (PD) is a complex disorder that involves multiple genetic and environmental factors. Several candidate genes have been speculated to be involved in the development of PD. We conducted a case-control study to investigate the association between PD and single nucleotide polymorphisms (SNP) at the *OPRM1*, *DAT*, *COMT* and *DRD2* genes in a Taiwanese population. *Methods:* The study included 260 PD cases and 100 healthy controls. SNPs at *OPRM1* (rs1799971), *DAT* (rs2652510 and rs2550956), COMT (rs4680) and DRD2 (rs1800497) were determined by high-resolution melting (HRM) analysis using a LightCycler®480 instrument. Each genotype was confirmed by direct sequencing. The genotype was checked for Hardy-Weinberg equilibrium. *Results:* The chi-square test (χ^2) was used to examine the association between SNP genotypes and PD. The *OPRM1* (rs1799971), *DAT* (rs2652510 and rs2550956), *COMT* (rs4680) and *DRD2* (rs1800497) polymorphisms showed no association with PD. Notably, an additional meta-analysis indicated that *DAT* rs2652510 was significantly associated with PD risk.
Conclusions: In the Taiwanese population the frequency of the *OPRM1*, *DAT*, *COMT*, and *DRD2* genotypes exhibits no difference between the PD patients and normal controls. The meta-analysis of the original and available data from published studies resulted in significant *p* values <0.01 for *DAT* rs2652510.

INTRODUCTION

Parkinson's disease (PD) is the second most common age-related neurodegenerative disease.[1] PD is a chronic, progressive, and neurodegenerative movement disorder that is characterized by a degeneration of dopaminergic neurons of the pars compacta of the substantia nigra, leading to the loss of dopamine in the striatum. PD is a complex disorder that involves multiple genetic and environmental factors.[2,3]

Pathological features of classical PD comprise the selective degeneration of pigmented dopaminergic neurons in the ventral substantia nigra, the presence of ubiquitinated protein deposits in the cytoplasm of neurons (Lewy bodies) and within neuritis (Lewy neuritis).[4] Several genes involved in dopamine metabolism and transporting, exogenous or endogenous toxin metabolism, iron homeostasis, inflammation, and mitochondrial abnormalities might play a role in the pathogenesis of PD.[5] Recently, research conducted in the field of pharmacogenetics has indicated that the genetic variability of each individual determines to a considerable extent the inter-individual variability in response to drug treatment in PD.[6] At present, many genetic association studies of PD have been reported in literatures. However, these studies have provided inconsistent or negative results. This may be due to the PD involving multiple genes with small effects and their interactions with each other as well as with non-genetic events.[7,8] Molecular genotyping of PD has led to the discovery of candidate genes involved in PD susceptibility. In fact, the primary causes of the disease remain uncertain. Therefore, there is a need to identify candidate

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gene polymorphisms that may be associated with the susceptibility to PD.

Dopamine is one of the major neurotransmitters that is important for the control of motor and cognitive functions, and it exerts its effect through interactions with receptors.[9] The dysfunction of dopaminergic neurotransmission in the central nervous system is associated with the development of PD.[10] The dopamine transporter (DAT) is an important molecule for movement as well as for Parkinsonism[11], and it plays a central role in the spatial and temporal buffering of released dopamine and key roles in its recycling. DAT acts in order to terminate dopaminergic neurotransmission by the reaccumulation of dopamine into presynaptic neurons. According to its central role in dopaminergic neurotransmission, *DAT* has been demonstrated as a candidate gene for PD, however with largely equivocal results. Dopamine D2 receptor (DRD2) is a potential candidate gene for susceptibility to PD, because it is relevant with locomotor function for D2-null mice that exhibit a parkinsonian-like phenotype.[12] DRD2 and Dopamine D4 receptor (DRD4) have demonstrated that both receptors govern the signaling effect and modulate the motor behavior and activity of nigrostriatal neurons.[13] Genetic variations in these proteins may influence the susceptibility to PD, because they are responsible for dopaminergic neurotransmission.[14] Catechol-O-methyltransferase (COMT) is an enzyme that by methylation inactivates neurotransmitters and toxic catechols such as the immediate precursor of dopamine. Decreased COMT activity may contribute to the increased metabolism of dopamine to neuromelanin, and it can enhance the formation of cytotoxic radicals which contribute to neuronal degeneration.[15] Mu-opioid receptors (OPRM1) are located throughout extended brain circuits involved in positive reinforcement and are critical in processing reward, analgesia, and stress responses. It is a primary site action for many endogenous opioid peptides, including -endorphin and enkephalin.[16] OPRM1 also plays a role in drug addiction such as cocaine, nicotine, and alcohol.[17] In addition, a disorder of the opiate system may cause brain degenerative diseases. The functional significance of OPRM1 in the etiology of PD is not clear at this time.

The aim of this study was to investigate the association of OPRM1 (rs1799971), DAT (rs2652510 and rs2550956), COMT (rs4680 and rs4818), and DRD2 (rs1800497) in the Taiwanese population with PD by high-resolution melting (HRM) analysis.

METHODS

Subjects

Two hundred and sixty patients of Parkinson who visited Movement Disorder's Clinic between January 2009 and September 2010 at the Department of Neurology, Kaohsiung Medical University Hospital were enrolled in this study. Inclusion criteria included at least two of the three following symptoms of rigidity, bradykinesia, resting tremor, and a good response to levodopa. Patients who refused to cooperate or serious cormobidity in medical problems were excluded in this study. The patients included 137 men and 123 women ranging in age from 26 to 97 years (71.4±0.78). The control group consisted of 100 age-matched, healthy, non-PD subjects, and they were not taking any medication during the course of the study. All patients and controls were of the same race, ethnicity and geographic stratification. The diagnosis of idiopathic PD was made according to the UK Parkinson's Disease Society Brain Bank Clinical Diagnostic Criteria.[18] Clinical stage of the disease was evaluated according to the classification of Hoehn and Yahr.[19] This study was approved by the Institutional Review Board (IRB) of the Kaohsiung Medical University Hospital (KMUH-IRB-20110078). Written informed consents were obtained for each participant involved in the study.

Blood sampling

The EDTA blood samples were collected from 260 Parkinson patients and 100 control subjects. Genomic DNA was isolated from peripheral blood leukocytes using the salting out method which was standardized in the QIAamp® mini blood kit (QIAGEN, Valencia, CA). Genomic DNA was stocked at -20ºC before use.

HRM analysis

The set of primers for HRM, specific for all polymorphisms, were designed while fulfilling the requirements of the LightCycler® 480 System Gene Scanning Assay. All the amplicons were designed smaller than 300bp. In this study, the five pairs of primers for HRM analysis were newly selected using Primer3 software (http://frodo.wi.mit.edu/). Appropriate primers were named *H1-H10* as shown in Table 1. All the primers synthesized were of standard molecular biology quality (Protech Technology Enterprise Co., Ltd, Taiwan).

Table 1: Primers use for HRM analysis of *ORRM1*, *DAT*, *COMT* and *DRD2* polymorphisms

Detection for	Sequence (5' to 3')	Length of PCR amplicon (bp)	Annealing Temp. (°C)
ORRM1	H1 5'-GAAAAGTCTCGGTGCTCCTG-3' (forward) H2 5'-CAGTCCCTCCATGATCACG-3' (reverse)	266	62
DAT	H3 5'-CGCTTTCTCTGTGTCATCCA-3' (forward) H4 5'-TGACCTCTAGTCCCTGCACC-3' (reverse)	263	62
COMT	H5 5'-GGGCCTACTGTGGCTACTCA-3' (forward) H6 5'-GGCCCTTTTTCCAGGTCTG-3' (reverse)	269	60
DRD2	H7 5'-CCTTGCCCTCTAGGAAGGAC-3' (forward) H8 5'-GAGGAGCACCTTCCTGAGTG-3' (reverse)	205	60

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was tested for control subjects (20) using the chi-square test with one degree of freedom. To test for differences in SNP genotype frequencies between Parkinson's cases and controls, a chi-square test (χ^2) was used. The strength of the association between Parkinson's disease and SNPs was determined by the standard odd ratio (OR) equation. The t test was used to compare differences in the means of continuous variables with $p < 0.05$ considered statistically significant. Statistical analysis was calculated using SPSS software. Power analysis was performed using the software available at http://pngu.mgh.harvard.edu/~purcell/gpc/.

RESULTS

HRM analysis and genotyping

A total of 5 SNP loci were identified by the HRM analysis. To optimize the HRM analysis for each SNP, control DNA was derived from the general population. Each SNP was analyzed using the 96-well LightCycler® system from 100 DNA templates from normal subjects and 260 DNA templates from those with Parkinson's disease. Using HRM conditions as listed in Table 1, the 5 SNPs were identified on the basis of a clear separation of differentially shifted melting curves (Figure 1A-1D). All cases showing melting curves divergent from each other underwent direct DNA sequencing in order to confirm the HRM results. In this study, we easily and accurately extended the application of HRM analysis for the genotyping of *ORRM1*, *DAT*, *COMT*, and *DRD2* using the

HRM analysis. We selected 15 samples for direct sequencing in each run in order to confirm the genotype.

Statistics

The frequency of genotypes for the five known polymorphism studies conducted on both controls and PD patients is shown in Table 2. There was no significant deviation from the HWE in the two groups of subjects studied, with regard to these polymorphisms. The study had limited power to detect potentially small effects of these SNPs on PD, which is a complex disorder involving multiple genetic and environmental factors. Therefore, larger sample sizes of subjects in each group would be required.

For SNP rs1799971 at the *OPRM1* gene, the OR for PD was 0.67 ($P = 0.21$) for the GG genotype and 0.82 ($P = 0.48$) for the AG genotype when compared with the reference AA genotype (Table 2). For SNP rs2652510 at the *DAT* gene, the OR for PD was 0.66 ($P = 0.11$) for the AG genotype and 1.71 ($P = 0.62$) for the GG genotype when compared with the reference AA genotype (Table 2). For SNP rs2550956, the OR for PD was 0.70 ($P = 0.19$) for the CT genotype and 0.69 ($P = 0.76$) for the CC genotype when compared with the reference TT genotype (Table 2). For SNP rs4680 at the *COMT* gene, the OR for PD was 0.59 ($P=0.05$) for the AG genotype and 0.88 ($P = 0.67$) for the AA genotype when compared with the reference GG genotype (Table 2). For SNP rs1800497 at the *DRD2* gene, the OR for PD was 1.11 ($P = 0.66$) for the CT genotype and 1.82 ($P=0.18$) for the TT genotype when compared with the reference CC genotype

Table 2: Association analyses of polymorphism genotyped in PD cases and controls

| Gene	SNP	Genotype	Case	%	Control	%	Adjusted OR (95% CI)	P value	HWE (P value)
OPRM1	rs1799971	AA	85	32.7%	27	27%	1		0.3
		AG	116	44.6%	45	45%	0.82 (0.47-1.42)	0.48	
		GG	59	22.7%	28	28%	0.67 (0.36-1.25)	0.21	
DAT	rs2652510	AA	196	75.7%	67	67%	1		0.2
		AG	58	22.4%	32	32%	0.66 (0.39-1.11)	0.11	
		GG	5	1.9%	1	1%	1.71 (0.20-14.89)	0.62	
	rs2550956	TT	200	77.2%	69	69%	1		0.3
		CT	57	22%	30	30%	0.70 (0.41-1.19)	0.19	
		CC	2	0.8%	1	1%	0.69 (0.06-7.73)	0.76	
COMT	rs4680	GG	100	38.46%	30	30%	1		0.4
		GA	90	34.62%	46	46%	0.59 (0.34-1.01)	0.05	
		AA	70	26.92%	24	24%	0.88 (0.47-1.62)	0.67	
DRD2	rs1800497	CC	120	46.2%	51	51%	1		0.7
		CT	110	42.3%	42	42%	1.11 (0.69-1.81)	0.66	
		TT	30	11.5%	7	7%	1.82 (0.75-4.42)	0.18	

HWE: Hardy-Weinberg equilibrium
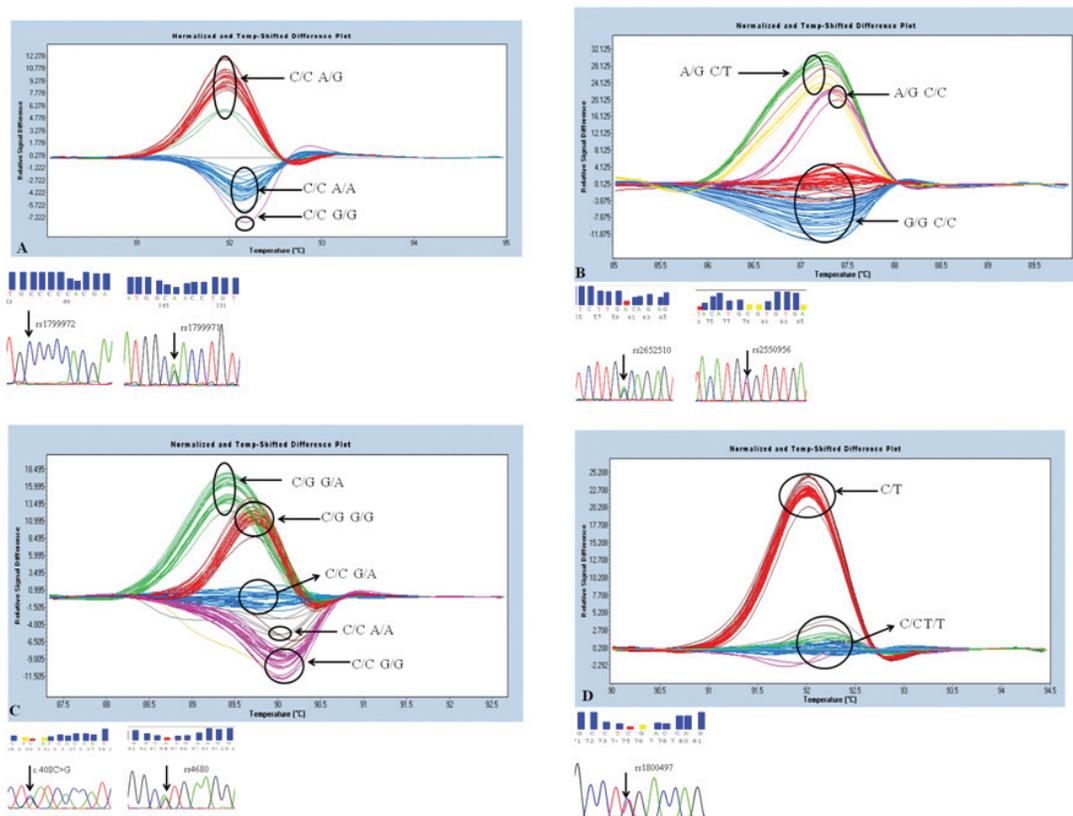
Figure 1A-1D. The genotyping of five SNPs was determined by HRM analysis and confirmed by direct DNA sequencing. Normalized plots and temperature shifted difference plots for the genotyping of five SNPs. (A) represents the melting profile of *OPRM1* (rs1799971). (B) represents the melting profile of *DAT* (rs2652510 and rs2550956). (C) represents the melting profile of *COMT* (rs4680). (D) represents the melting profile of *DRD2* (rs1800497).

(Table 2). Overall, the *OPRM1* (rs1799971), *DAT* (rs2652510 and rs2550956), *COMT* (rs4680) and *DRD2* (rs1800497) polymorphisms showed no association with PD.

Meta-analysis on the association between the 5 SNPs and PD risk

We conducted a meta-analysis of available data from published studies[14,21,25,26,29] on the association between 5 SNPs (rs1799971, rs2652510, rs2550956, rs4680, and rs1800497) and PD risk. As a result, we found that, overall, the pooled data showed that only *DAT* rs2652510 was significantly associated with PD risk (OR=0.7463; 95% CI=0.58-0.96; Figure 2). For the other 4 SNPs, rs1799971, rs2550956, rs4680, and rs1800497, was not significantly associated with PD risk (Figure 3).

DISCUSSION

In the current study, the candidate SNPs at the *ORRM1*, *DAT*, *COMT*, and *DRD2* polymorphisms showed no association with PD. However, our sample size does not provide a sufficient power to detect a mild effect on the development of PD. Based on the allele frequencies of the five SNPs in our cases, the best power estimate was 71% when a type I error rate was set to be 0.05 and an OR was set to be 2.0. For the less common SNPs (rs2652510 and rs2550956 at the DAT gene), the power can be as low as 10%. Therefore, the readers should take our conclusion with caution especially for the findings from these two less common SNPs.

We employed the HRM analysis for the SNP genotyping and it should be noted that the CC and TT genotypes at SNP rs1800497 of the *DRD2* gene cannot be differentiated in the normalized and temperature-shifted difference plots (Figure. 1D). This is due to the small melting
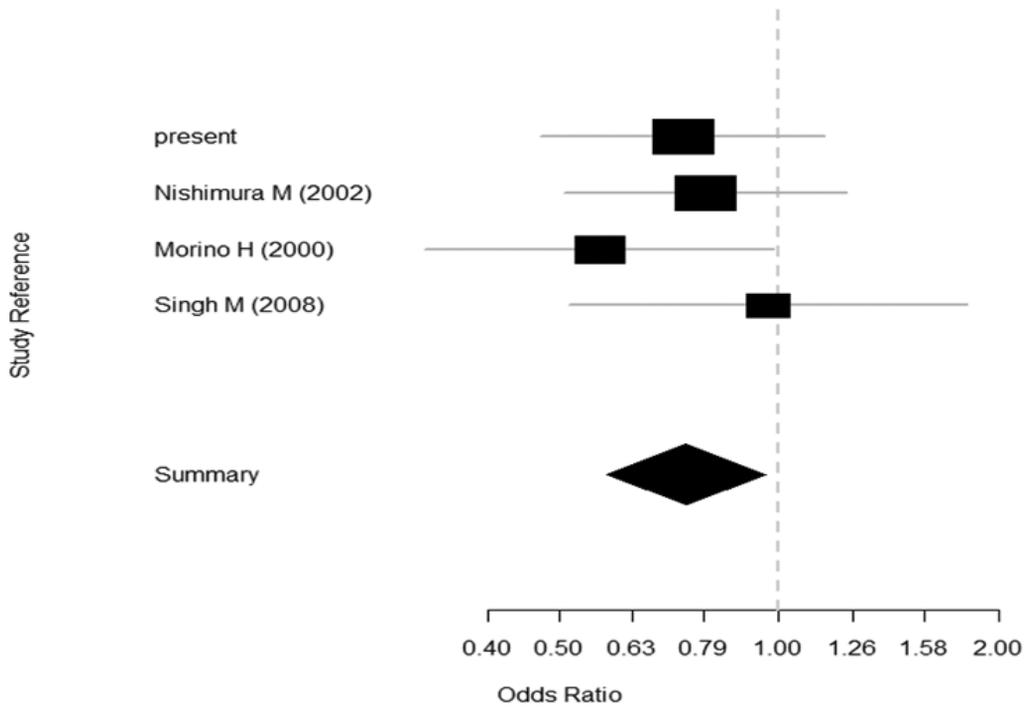
Figure 2. Meta-analysis of association between DAT rs2652510 and PD risk. OR and 95% CI were calculated using an allelic model for rs2652510.
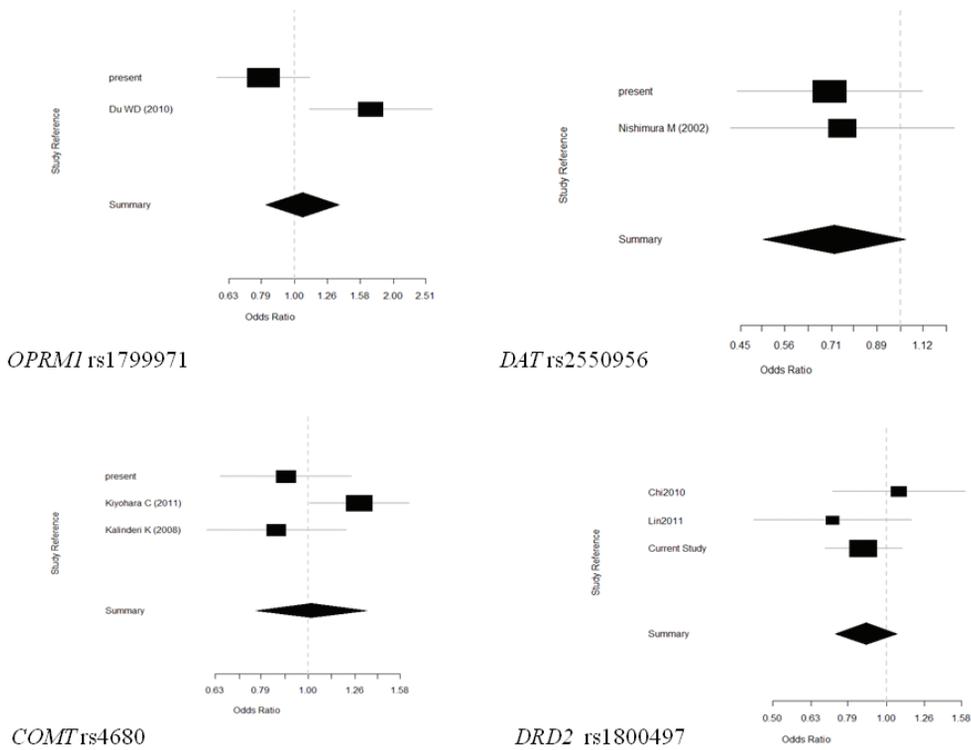

Figure 3. Meta-analysis of association between 4 SNPs (rs1799971, rs2550956, rs4680, and rs1800497) and PD risk. No significant association between 4 SNPs (rs1799971, rs2550956, rs4680, and rs1800497) and PD risk.
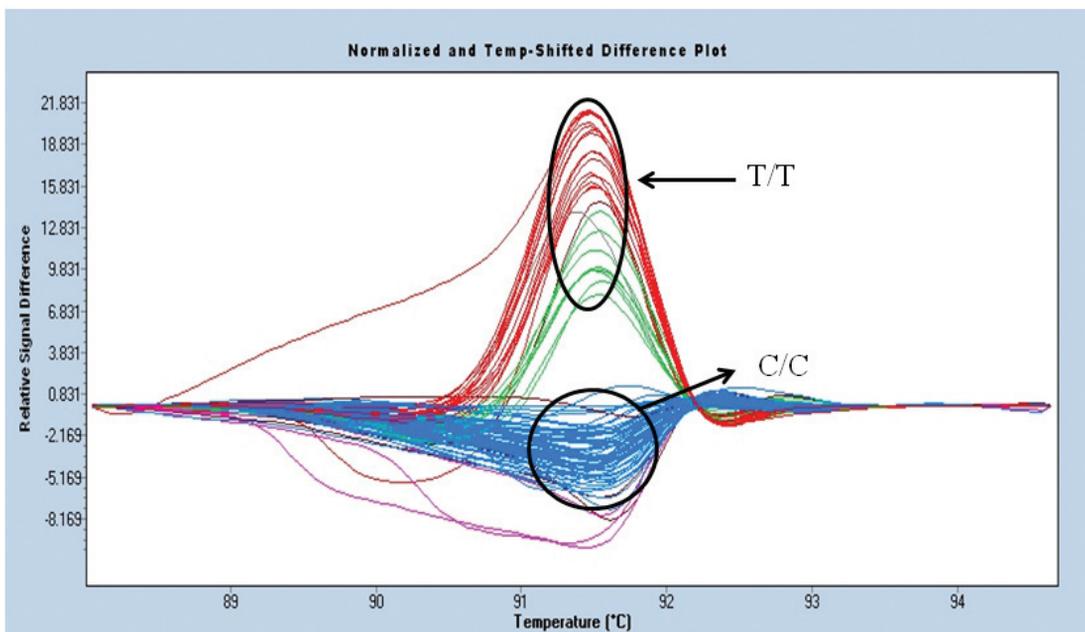
temperature (T_m) difference between the CC and TT genotypes (22). Therefore, all samples except the CG genotype in the normalized and temperature-shifted difference plots were chosen and mixed (1:1) with a known genotype CC, which was named *driver*. This strategy is used to differentiate between CC and TT genotypes. After mixing with driver, the melting profile of TT becomes heterozygous: however, the CC genotype remains the same in the normalized and temperature-shifted difference plots (Figure 4).

While serving as a primary target for opioid drugs and peptides, the OPRM1 mediates the effects of morphine and heroin, and OPRM1 also plays a role in the addiction to other drugs of abuse, such as cocaine, nicotine, and alcohol.[23] The A118G (rs1799971) and C17T (rs1799972) variants in exon 1 are prevalent SNPs that are located at the N terminus of the receptor, and their relationships to drug dependence, alcohol dependence, and related phenotypes were well studied earlier.[23] It is well established that receptors encoded by the mutated allele of the A118G SNP were reported as having a three-fold higher binding affinity for €-endorphin compared with the WT receptor in AV-12 cells transfected with the 116G cDNA.[24] This SNP has been extensively studied in human diseases and drug response. Notably, only one study[25] described the role of OPRM1 A118G in PD where the allele G was strongly associated with PD. However, we failed to replicate such an association.

The frequency of DAT 1215G allele has been reported to decrease in Japanese PD patients as compared with controls.[26,27] In contrast, Lin *et al*.[28] indicated that the DAT polymorphism (1215A/G) does not play a major role in the susceptibility to PD in a Taiwanese population, which is consistent with our findings. In the present study, we also demonstrated that the DAT polymorphism (rs2550956) is not associated with PD. It should be noted that, the difference in the results among races could have occurred as a result of methodological problems such as selection bias and/or analytical tools. In addition, the etiology and pathogenesis of PD may differ among races and environmental exposure.

Many studies investigated the correlation between the COMT genetic polymorphism and PD. However, the findings were inconsistent. Several studies showed no significant difference between the COMT genetic polymorphism in PD patients.[29,30] Other studies showed a lower frequency of the COMT[L/L] genotype in Polish and Japanese populations with PD.[31,32] Wu *et al*.[33] indicated that there was no correlation between

Supplementary data

Figure 4. This strategy is used to differentiate between CC and TT genotypes for SNP rs1800497 at the *DRD2* gene. Normalized plots and temperature-shifted difference plots indicate the genotyping of the CC and TT genotypes for SNP rs1800497 at the *DRD2* gene by mixing with a *driver*.

the COMT[L] genotype and PD in a Taiwanese population. Our findings suggested that the COMT polymorphism may not play a role in the susceptibility to PD. Since we only analyzed two SNPs, we cannot exclude the possibility of other COMT genetic variants associated with PD or PD-related phenotypes.

The DRD2 rs1800497 polymorphism is a candidate locus for PD due to its influence on dopamine regulation[34], and the A1 allele is associated with a higher risk of motor fluctuations.[35] Two studies implicated that the T allele of DRD2 rs1800497 was associated with PD.[36,37] However, no significant association between DRD2 rs1800497 and DRD4 rs1800955 were reported in the Japanese population.

In summary, the *OPRM1*, *DAT*, *COMT* and *DRD2* genotypes shows no difference between the PD patients and normal controls in Taiwanese population. Intriguingly, this study discovered an association of *DAT* rs2652510 with PD risk by meta-analysis. However, more comprehensive studies and larger cohorts of subjects are necessary to conclusively determine an association of these SNPs with PD.

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DISCLOSURE

Conflict of Interest: None

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