パン類	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
食パン	1	2	3	4	5	6	7	8	1. 1枚以下 2. 2枚 3. 3枚以上
全粒パン，ライ麦パン	1	2	3	4	5	6	7	8	1. 1枚以下 2. 2枚 3. 3枚以上
ロールパン，フランスパン	1	2	3	4	5	6	7	8	1. 1個以下 2. 2個 3. 3個以上
あげパン，ドーナツ	1	2	3	4	5	6	7	8	1. 1個以下 2. 2個 3. 3個以上
その他のパン（菓子パン，レーズンパンなど）	1	2	3	4	5	6	7	8	1. 1個以下 2. 2個 3. 3個以上
ホットケーキ，フレンチトーストなど	1	2	3	4	5	6	7	8	1. 1枚以下 2. 2枚 3. 3枚以上
野菜サンド	1	2	3	4	5	6	7	8	1. 2切れ以下 2. 3～5切れ 3. 6切れ以上
肉・ハムサンド	1	2	3	4	5	6	7	8	1. 2切れ以下 2. 3～5切れ 3. 6切れ以上
その他（卵，チーズ，魚など）のサンドイッチ	1	2	3	4	5	6	7	8	1. 2切れ以下 2. 3～5切れ 3. 6切れ以上

パンにつけるもの	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
バター	1	2	3	4	5	6	7	8	1. うすくぬる 2. 厚くぬる
マーガリン	1	2	3	4	5	6	7	8	1. うすくぬる 2. 厚くぬる
ピーナツバター	1	2	3	4	5	6	7	8	1. うすくぬる 2. 厚くぬる
ジャム，はちみつ，シロップ	1	2	3	4	5	6	7	8	1. うすくぬる 2. 厚くぬる

牛乳，チーズ	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
牛乳（そのままで，またはコーンフレークなどにかけて）	1	2	3	4	5	6	7	8	1. コップ半分 2. コップ1杯 3. コップ2杯以上
低脂肪牛乳，ヨーグルト（そのままで，またはコーンフレークなどにかけて）	1	2	3	4	5	6	7	8	1. コップ半分 2. コップ1杯 3. コップ2杯以上
スキムミルク（そのままで，またはコーンフレークなどにかけて）	1	2	3	4	5	6	7	8	1. コップ半分 2. コップ1杯 3. コップ2杯以上
ココア	1	2	3	4	5	6	7	8	1. コップ半分 2. コップ1杯 3. コップ2杯以上
乳飲料（カルピス，ヤクルトなど）	1	2	3	4	5	6	7	8	1. コップ半分 2. コップ1杯 3. コップ2杯以上
チーズ	1	2	3	4	5	6	7	8	1. 半切れ 2. 1切れ 3. 2切れ以上

デザート，スナック	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
アイスクリーム	1	2	3	4	5	6	7	8	1. 半カップ以下 2. 1カップ 3. 2カップ以上
シャーベット，アイスミルク かき氷など	1	2	3	4	5	6	7	8	1. 半カップ以下 2. 1カップ 3. 2カップ以上
クッキー，ビスケット	1	2	3	4	5	6	7	8	1. 1～2枚 2. 3～4枚 3. 5枚以上
ケーキ	1	2	3	4	5	6	7	8	1. 半個（小さいもの1個） 2. 1個 3. 2個以上
パイ（レモンパイ，アップルパイなど）	1	2	3	4	5	6	7	8	1. 半切れ 2. 1切れ 3. 2切れ以上
プリン	1	2	3	4	5	6	7	8	1. 1個以下 2. 2個 3. 3個以上
カステラ	1	2	3	4	5	6	7	8	1. 半切れ 2. 1切れ 3. 2切れ以上
チョコレート，チョコレート菓子	1	2	3	4	5	6	7	8	1. 1～2個 2. 3～4個 3. 5～6個
まんじゅう，ようかん	1	2	3	4	5	6	7	8	1. 1個以下 2. 2個 3. 3個以上

デザート，スナック	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
クラッカー，あられ，おかきせんべい，カッパエビセンなど	1	2	3	4	5	6	7	8	1. せんべいにして1枚以下 2. 2枚 3. 3枚以上
だんご	1	2	3	4	5	6	7	8	1. 串だんごにして1本以下 2. 串だんご2本 3. 串だんご3本以上
ナッツ（ピーナツ，カシューナッツなど）	1	2	3	4	5	6	7	8	1. 10粒以下 2. 1/4カップ 3. 半カップ以上
ポテトチップ，トンガリコーンなど	1	2	3	4	5	6	7	8	1. 小袋（1/2カップ位）以下 2. 1カップ 3. 大袋の半分以上
ポップコーン	1	2	3	4	5	6	7	8	1. 1～3カップ 2. 4～5カップ 3. 6カップ以上

つけもの	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
たくあん	1	2	3	4	5	6	7	8	1. 1切れ 2. 2～4切れ 3. 5切れ以上
かぶらづけ しなづけ	1	2	3	4	5	6	7	8	1. 1切れ 2. 2～4切れ 3. 5切れ以上
みそづけ しょうゆづけ	1	2	3	4	5	6	7	8	1. 1切れ 2. 2～4切れ 3. 5切れ以上
一夜づけ，即席づけ（きゅうり，なす）	1	2	3	4	5	6	7	8	1. 小皿半分 2. 小皿1杯 3. 小皿2杯以上
白菜，水菜のつけもの	1	2	3	4	5	6	7	8	1. 小皿半分 2. 小皿1杯 3. 小皿2杯以上
梅ぼし，らっきょ	1	2	3	4	5	6	7	8	1. 1個 2. 2～4個 3. 5個以上
その他のつけもの	1	2	3	4	5	6	7	8	1. 小皿半分 2. 小皿1杯 3. 小皿2杯以上

食卓で使う調味料	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
塩	1	2	3	4	5	6	7	8	1. 1振り 2. 2～3振り 3. 4振り以上
しょうゆ	1	2	3	4	5	6	7	8	1. 小さじ半分 2. 小さじ1杯 3. 小さじ2杯以上
酢，ぽん酢	1	2	3	4	5	6	7	8	1. 大さじ半分 2. 大さじ1杯 3. 大さじ2杯以上
ソース	1	2	3	4	5	6	7	8	1. 大さじ半分 2. 大さじ1杯 3. 大さじ2杯以上
ドレッシング	1	2	3	4	5	6	7	8	1. 大さじ半分 2. 大さじ1杯 3. 大さじ2杯以上
ノンオイルのドレッシング	1	2	3	4	5	6	7	8	1. 大さじ半分 2. 大さじ1杯 3. 大さじ2杯以上
マヨネーズ	1	2	3	4	5	6	7	8	1. 大さじ半分 2. 大さじ1杯 3. 大さじ2杯以上
トマトケチャップ	1	2	3	4	5	6	7	8	1. 大さじ半分 2. 大さじ1杯 3. 大さじ2杯以上

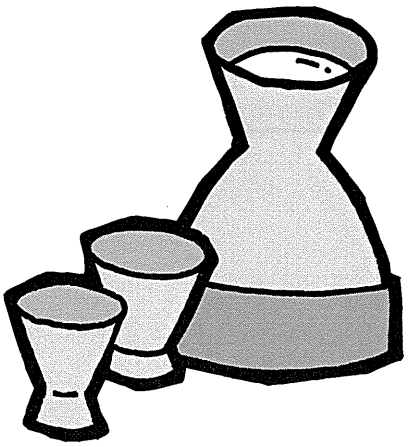
肉の脂（あぶら）の部分をどれ位食べましたか

1. 脂の部分は全部食べた
2. 脂の部分の一部を食べた
3. 脂の部分は食べなかった
4. 肉は全く～ほとんど食べなかった

鶏肉（かしわ）の脂（あぶら）の部分をどれ位食べましたか

1. 脂の部分は全部食べた
2. 脂の部分の一部を食べた
3. 脂の部分は食べなかった
4. 鶏肉は全く～ほとんど食べなかった

アルコール類	この1年間の平均回数								1回の量（平均）
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2回以上	
日本酒	1	2	3	4	5	6	7	8	1. 1合以下 2. 2　合 3. 3　合 4. 4合以上
しょうちゅう	1	2	3	4	5	6	7	8	1. 1合以下 2. 2　合 3. 3　合 4. 4合以上
ビール（生ビールを含む）	1	2	3	4	5	6	7	8	1. 缶ビール1本以下 2. 大びん1本 3. 大びん2～3本 4. 大びん4本以上
ライトビール	1	2	3	4	5	6	7	8	1. 缶ビール1本以下 2. 大びん1本 3. 大びん2～3本 4. 大びん4本以上
ワイン	1	2	3	4	5	6	7	8	1. 1杯以下 2. 2　杯 3. 3　杯 4. 4杯以上
ウィスキー，ブランデー，ジン，ウォッカ，ラム，カクテルなど	1	2	3	4	5	6	7	8	1. 1杯以下 2. 2　杯 3. 3　杯 4. 4杯以上



その他の飲み物	この1年間の平均回数								
	全くほとんどなかった	1か月に1回	1か月に2～3回	1週間に1回	1週間に2～3回	1週間に4～6回	1日に1回	1日に2～3回	1日に4回以上
コーヒー（缶コーヒー，インスタントコーヒーを含む）	1	2	3	4	5	6	7	8	9
カフェイン抜きコーヒー	1	2	3	4	5	6	7	8	9
紅茶	1	2	3	4	5	6	7	8	9
緑茶	1	2	3	4	5	6	7	8	9
ウーロン茶	1	2	3	4	5	6	7	8	9
炭酸飲料（コーラ，キリンレモン，サイダーなど）	1	2	3	4	5	6	7	8	9
スポーツドリンク（ポカリスエットなど）	1	2	3	4	5	6	7	8	9

コーヒーまたは紅茶に砂糖などを入れましたか

1. 砂糖，はちみつを入れた
2. 人工甘味料（シュガーカットなど）を入れた
3. 上記のどれも入れなかった
4. コーヒー，紅茶は全く～ほとんど飲まなかった

コーヒーまたは紅茶にクリープなどを入れましたか

1. クリームを入れた
2. 牛乳（濃縮牛乳を含む）を入れた
3. 粉ミルク（クリープ）を入れた
4. 上記のどれも入れなかった
5. コーヒー，紅茶は全く～ほとんど飲まなかった

III. 研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Miyake Y, Tanaka K, Fukushima W, Kiyohara C, Sasaki S, Tsuboi Y, Oeda T, Shimada H, Kawamura N, Sakae N, Fukuyama H, Hirota Y, Nagai M, Nakamura Y, Fukuoka Kinki Parkinson's Disease Study Group.	*PARK16* polymorphisms, interaction with smoking, and sporadic Parkinson's disease in Japan.	J Neurol Sci	362	47-52	2016

Ⅳ．研究成果の刊行物・別刷

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PARK16 polymorphisms, interaction with smoking, and sporadic Parkinson's disease in Japan

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ABSTRACT

Epidemiological evidence on the relationships between *PARK16* single nucleotide polymorphisms (SNPs) and Parkinson's disease (PD) is inconsistent. We examined this issue in Japan. Included were 229 cases within six years of PD onset. Controls were 356 patients without neurodegenerative disease. Compared with subjects with the AA genotype of SNP rs823128, those with the AG genotype, but not the GG genotype, had a significantly reduced risk of sporadic PD. Compared with the AA genotype of SNP rs947211, both the AG genotype and the GG genotype were significantly related to an increased risk of sporadic PD. Using subjects with the AA genotype of SNP rs823156 as a reference group, there were significant inverse relationships under the additive and dominant models. No significant relationships were found between SNPs rs16856139 or rs11240572 and sporadic PD. The CAAAC, the TGAGA, and the CAGAC haplotypes were significantly related to sporadic PD. The additive interaction between SNP rs823128 and smoking affecting sporadic PD was significant, although the multiplicative interaction was not significant. The *PARK16* SNPs rs823128, rs947211, and rs823156 and the CAAAC, TGAGA, and CAGAC haplotypes may be significantly associated with sporadic PD in Japan. New evidence of an additive interaction between SNP rs823156 and smoking is suggested.

1. Introduction

PARK16, located on 1q32, which contains five genes (*SLC45A3*, *NUCKS1*, *RAB7L1*, *SLC41A1*, and *PM20D1*) within 169.6 kb, was identified as a new Parkinson's disease (PD) susceptibility locus in a genome-wide association study (GWAS) in the Japanese population in 2009. The association between PD and each of seven single nucleotide polymorphisms (SNPs) (rs16856139, rs823128, rs823122, rs947211, rs823156, rs708730, and rs11240572) surpassed genome-wide significance [1]. In a GWAS in Caucasians, the relationships between SNPs rs823128, rs823156, or rs11240572 and PD did not reach genome-wide significance: the minor allele frequencies of these SNPs in controls were 0.04, 0.18, and 0.04, respectively, though the association with SNP rs823128 was significant among combined samples from stage I and stage II [2]. Two GWAS conducted in the UK and US found no associations between *PARK16* SNPs and PD [3,4]. *PARK16* was not included in the top 57 candidate SNPs in a GWAS in the Ashkenazi Jewish population [5]. Several genetic association studies [6–19] have investigated the relationships between *PARK16* SNPs and PD, but these studies have produced mixed findings. A 2012 meta-analysis using the PDGene

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database reported evidence for genome-wide significance, showing that SNP rs947211 was significantly associated with PD in Caucasian populations, while SNP rs823156 was significantly associated with PD in Asian populations [20].

To contribute to the body of available evidence regarding the association between *PARK16* SNPs and the risk of sporadic PD, we examined this issue using data from a multicenter hospital-based case–control study in Japan. In addition, we conducted haplotype analyses and investigated the possibility of interaction between the SNPs and smoking, which is known to be inversely related to PD.

2. Methods

2.1. Study population

PD cases were recruited at three university hospitals and one national hospital in Fukuoka Prefecture, on the island of Kyushu in southern Japan, and at three university hospitals, three national hospitals and one municipal hospital in Osaka, Kyoto, and Wakayama Prefectures, all of which are in the Kinki region, located in the mid-western part of the mainland. Eligible cases were patients who were within six years of the onset of PD and who had been diagnosed by one of the collaborating neurologists at one of the 11 collaborating hospitals according to the United Kingdom PD Society Brain Bank clinical diagnostic criteria [21]. The neurologists in charge asked their eligible PD patients to participate in our case–control study. Of 298 eligible PD cases identified during the period between April 1, 2006 and March 31, 2008, 250 agreed to participate in the study (response rate: 84%).

In the same time period, control subjects were recruited from departments other than neurology (orthopedic surgery, ophthalmology, otorhinolaryngology, plastic surgery, and oral surgery) at three of the 11 collaborating hospitals: one university hospital in Fukuoka Prefecture and one university hospital and one national hospital in the Kinki region. Control subjects were not matched to cases, either individually or in larger groups. Control candidates, who were inpatients or outpatients without neurodegenerative diseases at any of these three hospitals, were approached by an attending doctor or by one of our research nurses to participate in our case–control study. Eventually, 372 control candidates participated in our study whereas 156 refused (response rate: 70%).

Of the 250 cases and 372 control subjects who participated in our study, 240 cases and 371 controls gave informed consent to genotyping. Excluded were 11 cases and 12 controls with a family history of PD, one control with missing data on smoking, one control with missing data on caffeine intake, and one control with missing data on SNPs because genotype identification was impossible. The final analysis thus comprised 229 cases and 356 control subjects. The ethics committees of the 11 collaborating hospitals (Fukuoka University, Utano National Hospital, Osaka City University, Kyushu University, Wakayama Medical University, Kyoto University, Kurume University, Minami-Kyoto National Hospital, Toneyama National Hospital, Kyoto City Hospital, and National Omuta Hospital) approved our case–control study. Written informed consent was obtained from all subjects.

2.2. Questionnaire

Participants filled out a set of two self-administered questionnaires and mailed these materials to the data management center or handed them to research nurses. Our research technicians completed missing answers and/or illogical data by telephone or in-person interview.

Dietary habits during the preceding month were assessed using a self-administered, semi-quantitative, comprehensive diet history questionnaire. Reported intake levels of coffee, black tea, and Japanese and Chinese teas were used to estimate caffeine intake. Energy-adjusted intake was calculated according to the density method. A second questionnaire elicited information on sex, age, smoking habits, and family history of PD. A history of smoking was defined as having smoked at least once per day for at least one year.

2.3. DNA extraction and genotyping

Genomic DNA from buccal specimens collected with BuccalAmp swabs (Epicenter BioTechnologies, Madison, WI, USA) was extracted using a QIAmp DNA mini kit (Qiagen, Inc., Valencia, CA, USA). Five *PARK16* SNPs [rs16856139 (*SLC45A3*), rs823128 (*NUCKS1*), rs947211 (Intergenic), rs823156 (*SLC41A1*), and rs11240572 (*PM20D1*)] were genotyped using TaqMan SNP Genotyping Assays on the StepOnePlus machine (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Of the seven SNPs identified in a 2009 Japanese GWAS [1], SNPs rs708730 and rs823122 were excluded in a case–control study conducted in Singapore because r^2 between rs708730 and rs823156 and r^2 between rs823122 and rs823128 > 0.8 [6]; this finding prompted us to exclude the two SNPs from the present study as well.

2.4. Statistical analysis

Departures from the Hardy–Weinberg equilibrium were assessed among the control subjects using the chi-square test. Linkage disequilibrium was examined using Haploview software version 4.2 (Broad Institute, Cambridge, MA, USA) [22]. Logistic regression analysis was performed to calculate the crude odds ratios (ORs) and 95% confidence intervals (CIs) for sporadic PD relative to the SNPs under study, with the reference category being the homozygote of the major allele among the control subjects. Multiple logistic regression analysis was used to control for sex, age, region of residence, smoking, and caffeine intake. Smoking and caffeine intake were inversely associated with PD in this population [23,24]. The statistical power calculation was performed using QUANTO version 1.2 [25]. Haplotypes and their frequencies were inferred according to the expectation maximization algorithm. For differences in haplotype frequency between the cases and control subjects, crude ORs and 95% CIs were calculated based on the frequency of each haplotype relative to all other haplotypes combined. We examined multiplicative and additive interactions between *PARK16* rs823128 and smoking with regard to the risk of sporadic PD. Multiplicative interaction was estimated by introducing a multiplicative term into a multiple logistic regression model. Three measures for the additive interaction were calculated using the Excel sheet provided by Andersson et al. [26]: 1) relative excess risk due to interaction (RERI), 2) attributable proportion due to interaction (AP), and 3) synergy index (S). RERI is the excess risk due to an interaction relative to the risk without exposure. AP refers to the attributable proportion of disease among individuals exposed to both factors that is due to the factors' interaction. S is the excess risk from both exposures when there is an additive interaction, relative to the risk from both exposures without an interaction. RERI = 0, AP = 0, or S = 1 means no interaction or strict additivity; RERI > 0, AP > 0, or S > 1 means positive interaction or more than additivity; RERI < 0, AP < 0, or S < 1 means negative interaction or less than additivity [27]. If any of the null values (0 in RERI and AP or 1 in S) falls outside the 95% CI of its respective measurement, then the additive interaction is considered statistically significant. Excluding the calculation of linkage disequilibrium and statistical power calculation, all statistical analyses were performed using STATA/SE software version 13.1 (StataCorp, College Station, TX, USA).

3. Results

Compared with control subjects, cases were more likely to be older and non-smokers and to report a low caffeine intake (Table 1). There were no differences between cases and controls with regard to sex or region of residence.

Table 1
Characteristics of the study population.

Variable	n (%)		P-value
	Cases (N = 229)	Controls (N = 356)	
Sex			0.94
Male	88 (38.4)	138 (38.8)	
Female	141 (61.6)	218 (61.2)	
Age, years, mean ± SD	68.4 ± 8.7	66.6 ± 8.5	0.01
Onset age, years, mean ± SD	65.7 ± 8.8		
Region of residence			0.27
Fukuoka	86 (37.6)	150 (42.1)	
Kinki	143 (62.5)	206 (57.9)	
Ever smoked			0.001
No	167 (72.9)	212 (59.6)	
Yes	62 (27.1)	144 (40.5)	
Caffeine intake, mg/4184 kJ, mean ± SD	149.3 ± 110.8	193.7 ± 139.3	0.0001

Among the control subjects, deviation from Hardy–Weinberg equilibrium was not observed for any of the SNPs ($P = 0.16$ to 1.00). SNPs rs823128, rs947211, rs823156, and rs11240572 were in strong linkage disequilibrium with each other ($D' = 0.84$ to 0.98; Table 2).

In the multivariate model, compared with a reference group of subjects with the AA genotype of SNP rs823128, those with the AG genotype, but not the GG genotype, had a significantly reduced risk of sporadic PD: the adjusted OR (95% CI) for the AG genotype was 0.64 (0.42–0.97) (Table 3). Under the additive and dominant models, the inverse associations were significant: the adjusted ORs (95% CIs) were 0.64 (0.44–0.93) and 0.62 (0.41–0.93), respectively. Compared with the AA genotype of SNP rs947211, both the AG genotype and the GG genotype were significantly related to an increased risk of sporadic PD: the adjusted ORs (95% CIs) were 1.60 (1.01–2.53) and 3.06 (1.83–5.12), respectively. Under the additive and dominant models, the adjusted ORs (95% CIs) were 1.76 (1.36–2.28) and 1.99 (1.29–3.07), respectively. Using subjects with the AA genotype of SNP rs823156 as a reference group, neither the AG genotype nor the GG genotype was evidently associated with sporadic PD in the multivariate model, while significant inverse relationships were observed under the additive and dominant models: the adjusted ORs (95% CIs) were 0.68 (0.48–0.96) and 0.67 (0.46–0.98), respectively. There were no significant relationships between SNPs rs16856139 or rs11240572 and sporadic PD in any genetic model after adjustment for the confounders under study. With respect to SNP rs11240572, an inverse association fell just short of the significance level under the dominant model (adjusted OR [95% CI] was 0.70 [0.48–1.02], $P = 0.06$). The statistical power calculation revealed that, using our sample size, we could detect the gene-disease association for an OR of 0.588 with an accuracy of more than 80% at a significance level of 0.05 with a two-sided alternative hypothesis under the dominant model.

When haplotypes with a frequency of less than 1% in either cases or control subjects were excluded, seven haplotypes remained (Table 4). Given the haplotype order of rs16856139, rs823128, rs947211, rs823156, and rs11240572, the CAAAC and TGAGA haplotypes were significantly inversely related to sporadic PD compared with all other haplotypes combined: the crude ORs (95% CIs) were 0.64 (0.49–0.84) and 0.58 (0.35–0.96), respectively. In contrast, the CAGAC haplotype was significantly positively associated with sporadic PD in comparison with all other haplotypes combined: the crude OR (95% CI) was 1.78 (1.40–2.28).

Compared with subjects with the AA genotype of SNP rs823128 who had ever smoked, those with the AG or GG genotype who had never smoked had a 3.3-fold increased risk of sporadic PD: the additive interaction between SNP rs823128 and smoking affecting sporadic PD was significant because the 95% CIs of the RERI and AP values were >0, while the multiplicative interaction was not significant (Table 5).

Table 2
Pairwise linkage disequilibrium of *PARK16* polymorphisms (r^2 below and D' above the diagonal).

	rs16856139	rs823128	rs947211	rs823156	rs11240572
rs16856139		0.61	0.79	0.63	0.74
rs823128	0.32		0.98	0.92	0.88
rs947211	0.08	0.15		0.98	0.89
rs823156	0.26	0.67	0.20		0.84
rs11240572	0.35	0.59	0.17	0.69	

4. Discussion

In a study of 433 cases and 916 controls conducted in Singapore, the *PARK16* SNPs rs823128, rs947211, rs823156, and rs11240572, but not rs16856139, were significantly associated with PD [6] (Table 6). A significant relationship was found between SNP rs823128 and PD in a case–control study in Taiwan, while no such significant relationship was observed in case–control series from Tunisia, the US, Canada, Norway, Ireland, and Poland [8]. There were significant associations between SNP rs16856139, rs823128, and rs823156, but not rs11240572, and PD in a Chinese study of 636 cases and 510 control subjects [10]. SNP rs823128, but not rs947211, was significantly related to PD in a Chilean sample (169 cases and 195 controls) [11]. Significant associations were observed between five SNPs including rs947211 and PD in 720 cases and 642 controls, all of Ashkenazi Jewish origin [14]. In a Korean study of 1036 cases and 1208 controls, SNPs rs947211 and rs11240574, but not rs16856139 or rs823156, were significantly associated with PD [16]. There was a significant relationship between SNP rs947211, but not rs823128, rs823156, or rs11240572, and PD in a case–control study in Taiwan (497 cases and 500 controls) [17]. In a Chinese study of 1061 cases and 1066 controls, no material associations were found between SNPs rs823156 or rs11240574 and PD [19]. These findings are in partial agreement with our results.

No significant relationship was shown between SNPs rs823156 or rs947211 and PD in a Spanish study of 1445 cases and 1161 controls [12]. In a case–control study in China (226 cases and 230 controls), SNPs rs16856139 and rs11240572, but not rs823128, rs947211, or rs823156, were significantly related to PD [13]. There were no evident associations between SNPs rs947211 or rs823128 and PD in a Chinese study of 323 cases and 345 controls [18]. These findings are at variance with the current results. The inconsistency of these findings with our results may be at least partly explained by differences in the genetic backgrounds of the populations examined, definitions of PD, and statistical power. For example, the minor allele frequency of SNPs rs823128 and rs11240572 in Caucasians is lower than that in Asians. Li et al. showed that the linkage disequilibrium pattern of SNPs close to rs947211 was similar in Caucasians and Asians, including Chinese, Japanese, and Malay, while the linkage disequilibrium patterns around rs823128 and rs823156 differed between Caucasian and Asian populations [28].

SNP rs823156 is located on the *SLC41A1* gene. SLC41A1 is a cell-membrane-localized Mg^{2+} carrier, conducting the exchange of intracellular Mg^{2+} for extracellular Na^{+} [29]. Kolisek et al. demonstrated that the substitution p.A350V in SLC41A1 is a gain-of-function mutation leading to increased Mg^{2+} extrusion from the cell, which might result in chronic intracellular Mg^{2+}-deficiency, a condition that is found in various brain regions of PD patients [30]. SNP rs823128 is located on the *NUCKS1* gene. NUCKS1 (nuclear, casein kinase, and cyclin-dependent kinase substrate one) is a nuclear, DNA-binding and highly phosphorylated protein and a substrate for casein kinase 2, cyclin dependent kinase-1 and DNA-activated kinase in vitro and in vivo; based on these characteristics, it appears to be important for cell cycle progression [31]. Satake et al. found that SNP rs947211 was strongly associated with transcript levels of *NUCKS1* [1].

Our results regarding the CAGAC haplotype of rs16856139, rs823128, rs947211, rs823156, and rs11240572 are in partial

Table 3
Association between *PARK16* polymorphisms and sporadic Parkinson's disease in Japan.

SNP	Model	Genotype	*n* (%)		Crude OR (95% CI)	Adjusted OR (95% CI)[a]
			Cases ($N = 229$)	Controls ($N = 356$)		
rs16856139	Co-dominant	CC	183 (79.9)	270 (75.8)	1.00	1.00
		CT	46 (20.1)	79 (22.2)	0.86 (0.57–1.29)	0.89 (0.58–1.37)
		TT	0 (0.0)	7 (2.0)		
	Additive				0.74 (0.50–1.08)	0.75 (0.51–1.12)
	Dominant				0.79 (0.53–1.18)	0.81 (0.53–1.24)
rs823128	Co-dominant	AA	183 (79.9)	254 (71.4)	1.00	1.00
		AG	43 (18.8)	94 (26.4)	0.63 (0.42–0.95)	0.64 (0.42–0.97)
		GG	3 (1.3)	8 (2.3)	0.52 (0.14–1.99)	0.43 (0.11–1.69)
	Additive				0.66 (0.46–0.94)	0.64 (0.44–0.93)
	Dominant				0.63 (0.42–0.93)	0.62 (0.41–0.93)
rs947211	Co-dominant	AA	37 (16.2)	103 (28.9)	1.00	1.00
		AG	114 (49.8)	185 (52.0)	1.72 (1.10–2.67)	1.60 (1.01–2.53)
		GG	78 (34.1)	68 (19.1)	3.19 (1.94–5.25)	3.06 (1.83–5.12)
	Additive				1.79 (1.40–2.30)	1.76 (1.36–2.28)
	Dominant				2.11 (1.39–3.21)	1.99 (1.29–3.07)
rs823156	Co-dominant	AA	170 (74.2)	233 (65.5)	1.00	1.00
		AG	55 (24.0)	112 (31.5)	0.67 (0.46–0.98)	0.70 (0.47–1.03)
		GG	4 (1.8)	11 (3.1)	0.50 (0.16–1.59)	0.41 (0.12–1.37)
	Additive				0.68 (0.49–0.95)	0.68 (0.48–0.96)
	Dominant				0.66 (0.46–0.95)	0.67 (0.46–0.98)
rs11240572	Co-dominant	CC	166 (72.5)	230 (64.6)	1.00	1.00
		CA	58 (25.3)	118 (33.2)	0.68 (0.47–0.99)	0.69 (0.47–1.01)
		AA	5 (2.2)	8 (2.3)	0.87 (0.28–2.69)	0.84 (0.26–2.71)
	Additive				0.74 (0.53–1.03)	0.74 (0.53–1.04)
	Dominant				0.69 (0.48–0.995)	0.70 (0.48–1.02)

[a] Adjusted for sex, age, region of residence, smoking, and caffeine intake.

agreement with those of the previously cited Korean study in which the CGGAAC haplotype of rs16856139, rs4245718, rs947211, rs823156, rs708730, and rs11240572 was significantly positively associated with PD [16].

To our knowledge, the present study is the first to show a significant additive interaction between SNP rs823128 and smoking with respect to the risk of sporadic PD; the multiplicative interaction, however, was not significant.

Several methodological limitations of the current study should be recognized. First, given that data on family history of PD was self-reported, some proportions of sporadic PD might be misclassified.

Second, information on smoking was also self-reported.

Third, our control subjects were selected from 3 of the 11 collaborating hospitals at which cases were recruited. The results of a sensitivity analysis restricted to cases who were recruited from three hospitals associated with recruitment of control subjects ($n = 145$) were similar to those in the overall analysis: the adjusted OR under the dominant model was 0.62 (95% CI: 0.38–0.999) for rs823128, 1.75 (95% CI: 1.07–2.86) for rs947211, and 0.65 (95% CI: 0.42–1.02) for rs823156.

Fourth, the number of cases and control subjects was rather small for a valid genetic association study. The lack of significant relationships between SNPs rs16856139 or rs11240572 and sporadic PD might be attributable to insufficient statistical power.

Fifth, correction for multiple testing, an appropriate procedure in initial exploratory analyses, was not performed in the present study. As this is a hypothesis testing study and part of the present findings is a replication of previously published results, we think that correction for multiple testing would cause us to underestimate our results.

Sixth, although adjustment was made for some confounders, residual confounding effects could not be ruled out.

The present study suggests that the *PARK16* SNPs rs823128, rs947211, and rs823156 and the CAAAC, TGAGA, and CAGAC haplotypes were significantly associated with the risk of sporadic PD in our Japanese population. Also, new evidence of an additive interaction between SNP rs823156 and smoking affecting sporadic PD is suggested. More studies should be performed to confirm the authenticity of our results and to clarify the biological mechanisms underlying the observed interactions.

Table 4
Haplotype analysis of 5 *PARK16* polymorphisms associated with sporadic Parkinson's disease in Japan[a].

Haplotype[b]	*n* (%)		Crude OR (95% CI)[c]
	Cases ($2N = 458$)	Controls ($2N = 712$)	
CAAAC	111 (24.2)	237 (33.3)	0.64 (0.49–0.84)
CAAGC	6 (1.3)	11 (1.5)	0.85 (0.26–2.52)
CAAGA	8 (1.7)	15 (2.1)	0.83 (0.30–2.09)
CAGAC	260 (56.8)	302 (42.4)	1.78 (1.40–2.28)
CGAGA	22 (4.8)	32 (4.5)	1.07 (0.59–1.93)
TAAAC	5 (1.1)	9 (1.3)	0.86 (0.23–2.89)
TGAGA	25 (5.5)	64 (9.0)	0.58 (0.35–0.96)

[a] Rare haplotypes (frequency less than 1% in either cases or controls) were deleted.
[b] Haplotype order is rs16856139, rs823128, rs947211, rs823156, and rs11240572.
[c] Crude OR for each haplotype is relative to all other haplotypes combined.

Table 5
Interaction between *PARK16* SNP rs823128 and smoking history affecting sporadic Parkinson's disease in Japan.

Genotype	Ever smoked			
	Yes		No	
	No. cases/controls	Adjusted OR (95% CI)[a]	No. cases/controls	Adjusted OR (95% CI)[a]
AA	47/103	1.00	136/151	1.68 (0.75–3.77)
AG + GG	15/41	1.10 (0.54–2.22)	31/61	3.30 (1.61–6.74)
P for multiplicative interaction = 0.19				
Measures of additive interaction[b]				
Relative excess risk due to interaction (RERI) = 1.51 (95% CI: 0.21–2.81)				
Attributable proportion due to interaction (AP) = 0.46 (95% CI: 0.12–0.80)				
Synergy index (S) = 2.94 (95% CI: 0.52–16.58)				

[a] Adjusted for sex, age, region of residence, and caffeine intake.
[b] Statistically significant when the 95% CI of RERI >0, the 95% CI of AP > 0, or the 95% CI of S > 1, indicating additive interaction.

Table 6
Findings from other genetic association studies on the relations between *PARK16* polymorphisms and Parkinson's disease.

References	Country	No. of cases and controls	Relations between *PARK16* polymorphisms and Parkinson's disease				
			rs16856139	rs823128	rs947211	rs823156	rs11240572
[6]	Singapore	433 and 916	No	Significant	Significant	Significant	Significant
[7]	UK	453 and 483		No			No
[8]	Taiwan	403 and 348		Significant			
[8]	Tunisia	159 and 302		No			
[8]	USA	1916 and 1009		No			
[8]	Canada	420 and 331		No			
[8]	Norway	675 and 582		No			
[8]	Ireland	363 and 372		No			
[8]	Poland	347 and 348		No			
[10]	China	636 and 510	Significant	Significant		Significant	No
[11]	Chile	169 and 195		Significant	No		
[12]	Spain	1445 and 1161			No	No	
[13]	China	226 and 230	Significant	No	No	No	Significant
[14]	Israel	720 and 642			Significant		
[16]	Korea	1036 and 1208	No		Significant	No	Significant
[17]	Taiwan	497 and 500		No	Significant	No	No
[18]	China	323 and 345		No	No		
[19]	China	1061 and 1066				No	No

Conflict of interest

None of the authors had any personal or financial conflict of interest.

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Appendix A

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