

Original Article

Association analysis of HLA-DRA in Chinese patients with sporadic Parkinson's disease

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Abstract: Our aim is to explore the linkage between single nucleotide polymorphisms (SNPs) in human leukocyte antigen (HLA)-DRA and Parkinson's disease (PD). 542 sporadic PD patients and 674 healthy controls were recruited to investigate this association in the Chinese population by the screening of 15 SNPs in *HLA-DRA*, and the association of rs3129882 was further re-evaluated by performing meta-analysis and meta-regression analysis. No SNPs in *HLA-DRA* were significantly associated with PD in the Chinese patients. Although the rs3129882 allele-G frequency in the Caucasian population was lower than that in Chinese population, meta-analysis showed no association of rs3129882 allele-G with PD in the Chinese or Caucasian population. In consideration of the heterogeneity ($I^2=78.6\%$, $Q=66.33$, $p<0.000$), subgroup analysis was performed, revealing a significant association between rs3129882 and PD in the Caucasian patients from the genome-wide association studies (OR=1.22, 95% CI: 1.16, 1.27); however, this association was not consistently observed in the studies performed using other DNA sequencing techniques. Meta-regression analysis, which accounted for 58.3% of the heterogeneity, revealed that the impact of the sequencing technique used was significant ($p=0.027$) compared with ethnicity. Regression analysis of the Caucasian population further showed that the sequencing technique used contributed to 71.2% of the heterogeneity ($p=0.033$). Our data support the absence of a linkage between the risk loci in *HLA-DRA* and PD in Chinese patients. The different DNA sequencing techniques used in PD studies may largely account for the controversial conclusions concerning rs3129882.

Keywords: HLA-DRA, Parkinson's disease, DNA sequencing analysis

Introduction

Parkinson's disease (PD) is a neurodegenerative disease affecting the elderly that features resting tremor, muscle rigidity, bradykinesia, and gait abnormalities [7]. It is characterized by dopaminergic (DA) neuron degeneration accompanying protein accumulation and the activation of microglia [2]. Aging, genetic defects and environmental toxins are involved in DA neuron degeneration [7]. Sporadic PD, which accounts for the majority of PD patients (70%-85%), is associated with a number of different genetic variants [18]. A genome-wide association study (GWAS) has demonstrated that human leukocyte antigen (*HLA-DRA*) is

closely linked to sporadic PD in the Caucasian population [11], suggesting that single nucleotide polymorphisms (SNPs) in *HLA-DRA* may be clinical biomarkers for PD prediction.

HLA complex genes belong to three classes [29]. *hla-dr* belongs to HLA class II, which includes the *dra* and *drb* genes [29]. *hla-dr* encodes α and β chains that form DR molecules [11]. In PD patients and animal models, activated microglia express the HLA-DR antigen, which is widely distributed in the substantia nigra [1], and this antigen has been shown to be a potential marker of PD in activated microglia [22]. At present, the molecular mechanism of *hla-dra* in PD is still unclear. Investigations of

PD-susceptible polymorphisms have focused on the HLA-DRA region, in which rs3129882 allele-G has been reported to be a risk factor for PD in individuals of European and Iranian ancestry [11, 17]; however, rs3129882 allele-A may increase the risk of PD in Chinese individuals [10, 37]. Notably, several studies have reported discrepant results on rs3129882 in other PD populations, such as those in Japan, Switzerland and Netherlands [28, 31, 32, 36]. To date, the linkage between rs3129882 and PD is controversial. Thus, we conducted a case-control study by performing SNP genotyping analyses of the HLA-DRA gene in the Chinese population and re-evaluated the relationship between rs3129882 and PD by meta-analysis and meta-regression analysis.

Materials and methods

Clinical data

A total of 542 sporadic PD patients and 674 healthy controls were recruited from the PD Research Center and Healthcare Center of the First Affiliated Hospital of Sun Yat-sen University between 2008-2015. All patients were diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria for sporadic PD [14]. The mean age of the PD patients was 56.9 ± 17.2 years, and 320 patients were male and 222 were female. A total of 84 patients had early-onset PD (age of onset ≤ 50 years), and 458 had late-onset PD (onset > 50 years). The mean age of the healthy controls was 55.4 ± 12.5 years, of whom 462 were male and 212 were female. Informed consent was obtained from all subjects, who specified self-claimed membership in the Han ethnic population and residency in the Guangdong Province of China and denied a family migration history. This study followed the ethical guidelines and was approved by the Hospital Ethnic Committee.

DNA collection and SNP genotyping

Venous blood samples (5 mL) were collected from all subjects. DNA was extracted using a gDNA Kit (Tigen Biotech, China). The reference genomic DNA sequences were obtained from GenBank database. Direct DNA sequencing was performed to screen for variant PCR products of rs3129882, which spans 1000 kb in HLA-DRA. The sequences of the primers used for PCR are as follows: HLA-DRA-rs3129882-

F: GGAATTGCCAAGGTCAATCC, and HLA-DRA-rs3129882-R: TGGCTTTGTCCACAGCTATG. Capillary electrophoresis was conducted using an ABI 3730XL DNA Analyzer. DNA sequences were analyzed using Mutation Surveyor v2.2 software (Soft Genetics, USA) and GeneMapper 4.1 software (Applied Biosystems Co., Ltd, USA).

Meta-analysis

We identified all relevant articles by performing searches of PubMed/MEDLINE, EMBASE, Web of Science and Chinese National Knowledge Infrastructure (CNKI) with the following search terms: "rs3129882", "HLA-DRA", "human leukocyte antigen" and "Parkinson's disease", "Parkinson disease", and "Parkinson's disease". This search was updated until October 2015. The included studies were reviewed independently by four authors (MS.M, YS.X, ZH.W, and CC.S). The inclusion criteria were as follows: (i) randomized study with no selection bias; (ii) the selection of all subjects among healthy volunteers from Chinese and Caucasian populations; and (iii) the calculation of allele frequencies by the direct gene counting method after adjusting for deviation from Hardy-Weinberg equilibrium (HWE). The exclusion criteria were as follows: (i) insufficient amount of basic patient information and information on subject recruitment provided (e.g., gender, age, and smoking status); and (ii) the method used to calculate allele frequencies or the sequencing technique used was unspecified. The publications with the largest group sizes were selected for each cohort study. A flowchart of the study selection process is shown in [Supplemental Data 1](#). Stata-11.0 software (Stata Corp., College Station, TX, USA) was used for genotype analysis. The effects of heterogeneity were estimated using the I^2 value and Cochran's Q-statistic test [12, 26], and the Q-test with $p < 0.05$ or $I^2 > 50\%$ was used to determine whether heterogeneity existed in meta-analysis. Sensitivity analysis and Begg's funnel plots were used to assess the quality and consistency of the included studies and publication bias in meta-analysis, respectively. The random-effects meta-regression model was used to trace the sources of heterogeneity. It was hypothesized that two covariates, including ethnicity (categorical variables: Chinese and Caucasian) and the sequencing technique used (categorical variables: GWAS and other

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Table 1. Information on SNPs in HLA-DRA in the Chinese population

SNP	Position	Genotype counts		MA	MAF			p for HWE
		Cases	Controls		Cases	Controls	p	
rs3129881	6:32441707	496/46/0	600/74/0	C	0.042	0.053	0.159	0.132
rs9501628	6:32441722	528/14/0	650/24/0	A	0.013	0.017	0.334	0.638
rs3129882	6:32441753	242/240/60	277/324/73	A	0.332	0.340	0.392	0.130
rs9268657	6:32441879	182/258/102	216/326/132	A	0.426	0.426	0.570	0.651
rs16822599	6:32441885	433/108/1	556/116/2	T	0.101	0.087	0.297	0.125
rs16822602	6:32441920	472/70/0	604/69/1	C	0.065	0.051	0.212	0.502
rs17496549	6:32441931	526/16/0	646/28/0	C	0.015	0.020	0.269	0.582
rs6931646	6:32442004	270/214/58	320/294/60	T	0.304	0.299	0.886	0.518
rs6911419	6:32442010	276/208/58	332/290/52	C	0.299	0.285	0.723	0.299
rs16822610	6:32442067	472/70/0	607/66/1	G	0.065	0.049	0.134	0.566
rs16822614	6:32442176	476/66/0	612/60/2	A	0.061	0.046	0.144	0.682
rs6911777	6:32442219	444/92/6	550/112/12	T	0.095	0.061	0.653	0.029
rs16822616	6:32442306	532/8/0	654/20/0	C	0.007	0.014	0.088	0.696
rs16822618	6:32442310	424/112/6	510/152/12	T	0.114	0.127	0.228	0.863
rs3129883	6:32442360	374/123/45	466/170/38	C	0.196	0.178	0.381	0.000

Direct DNA sequencing analysis demonstrating the presence of 15 SNPs within 1000 kb of rs3129882 in HLA-DRA. MA refers to the minor allele. MAF denotes the minor allele frequency, and *p* values for MAF comparisons are shown for the case and control groups. The HWE *p* values are the *p* values for the Hardy-Weinberg equilibrium test for the control group.

techniques), may be associated with heterogeneity in the meta-regression model [4]. The log odds ratios (ORs) of the covariates were identified as regression coefficients and were used to evaluate the impacts of the covariates with 95% confidence intervals (CIs). Subgroup analysis was based on ethnicity and the sequencing technique used, and regression analysis was performed to verify the contribution of the variables using STATA 9.0.

Statistical analysis

All groups were tested for deviation from HWE using SHEsis software platform (<http://linkage.rockefeller.edu/ott/linkutil.htm>). Data processing and statistical analysis were performed using SPSS 13.0 (SPSS Inc., Chicago, IL). Genotypes for each SNP were determined by direct counting. Allele frequencies were calculated using the formula $(2 \times \text{no. of homozygotes} + \text{heterozygotes}) / (2 \times \text{no. of samples})$. Alleles were tested for deviation from HWE using SHEsis software platform (Yong, et al. 2005). Differences in the genotypic and allelic frequencies between the PD patients and healthy controls for each SNP were determined using the χ^2 test with continuity correction and Fisher's test. ORs and 95% CIs were calculated by non-restrictive logistic regression. To avoid

type I errors caused by multiple comparisons, the calculated *p*-values were corrected with Bonferroni correction to obtain *P_c* values. A *P_c* < 0.05 was considered statistically significant.

Results

Direct sequencing of 1000 kb surrounding rs3129882 in HLA-DRA

A total of 15 SNPs in the HLA-DRA region (rs3129881, rs9501628, rs3129882, rs9268657, rs16822599, rs16822602, rs17496549, rs6931646, rs6911419, rs16822610, rs16822614, rs6911777, rs16822616, rs16822618, and rs3129883) were identified within 1000 kb of rs3129882. All SNPs in the PD patients, healthy controls, and subjects in all gender-based and onset-age-based subgroups were tested for HWE ($df=1$, $p>0.05$), located at coding sequence. The power analysis results suggested that the sample size was sufficient for susceptibility analysis under the following conditions: relative risk (OR) ≥ 1.5 , disorder-related gene frequency = 0.30, $\alpha = 0.05$ and $1-\beta = 80\%$. Haplotype analysis was performed, revealing no significant differences in haplotypes between the PD and control groups. Additionally, no significant differences were found in the

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Table 2. A comparison of genotype and allele frequencies for rs3129882 in different population

Subjects	Genotype frequency (rs3129882)			Allele frequency (rs3129882)		
	GG/GA/AA	χ^2	P	G/A	χ^2	P
CHB	14/23/7	1.417	0.224	51/37	1.864	0.172
JPT	13/21/8	1.689	0.191	47/37	2.914	0.088
CEU	11/32/17	11.976	0.001	54/66	19.269	0.000
YRI	14/24/22	7.266	0.007	52/68	22.556	0.000
This study	277/324/73	-	-	878/470	-	-

The frequencies of rs3129882 in the CHB, JPT, CEU and YRI populations determined using HapMap database (<http://hapmap.ncbi.nlm.nih.gov/>). CHB: Han Chinese in Beijing, JPT: Japanese in Tokyo, CEU: Utah residents with ancestry from northern and western Europe, and YRI: Yoruba in Ibadan, Nigeria. The χ^2 test was performed using a 3×2 contingency table, in which the population in this study served as the reference.

allele frequencies at all 15 SNPs between the PD patients and healthy controls (**Table 1**). The sequencing results for the rs3129882 locus are presented in **Supplemental Data 2**. No significant association was found between the genotype frequency of rs3129882 and PD in the dominant (OR=0.937, 95%CI: 0.845, 1.039, P=0.214), recessive (OR=0.989, 95% CI: 0.840, 1.164, P=0.894) or co-dominant model (OR=1.070, 95% CI: 0.968-1.183, P=0.188).

Comparison of the genotype and allele frequencies of rs3129882 among four populations

Racial genetic polymorphisms may explain the differences in PD manifestation among patients [8, 25]. Thus, we collected genotype data from four populations from HapMap database and compared the genotype distributions of rs3129882. We found that the minor allele frequency (MAF) of rs3129882 was A in the Chinese and Japanese populations but that it was G in Utah (USA) residents with ancestry from northern and western Europe (CEU) and Yoruba in Ibadan (YRI). No significant differences in genotype or allele frequencies of rs3129882 were found between the 674 healthy Chinese individuals in our study and 44 Chinese individuals from Beijing (CHB) ($\chi^2=1.417$, $p=0.224$) or 42 Japanese individuals from Tokyo (JPT) ($\chi^2=1.689$, $p=0.191$). The Chinese population in our study had higher frequencies of rs3129882-G/G and allele-G than the CEU (0.411 vs. 0.183, $\chi^2=11.976$, $p<0.001$; 0.651 vs. 0.450, $\chi^2=19.269$, $p<0.000$; respectively) and YRI populations (0.411 vs.

0.233, $\chi^2=7.266$, $p<0.004$; 0.651 vs. 0.433, $\chi^2=22.556$, $p<0.000$; respectively) (**Table 2**).

Meta-analysis of rs3129882 in Chinese population

We conducted meta-analysis to evaluate the relationship of rs3129882 allele-G with PD in the Caucasian and Chinese populations. This systematic review included seven studies containing 4,889 sporadic PD patients and 6,167 controls from the Chinese population [3, 9, 10, 19, 36, 37] and seven studies containing 11,234 sporadic PD

patients and 12,544 controls from the Caucasian population [13, 20, 24, 27, 28, 32, 33]. Three of the studies on the Caucasian population were GWASs that performed sequencing, and the other studies were conducted using PCR, TaqMan and pyrosequencing techniques (**Table 3**). Meta-analysis showed that rs3129882 allele-G had no association with PD (OR=1.02, 95% CI: 0.97, 1.12) and that it presented significant heterogeneity ($I^2=78.6\%$, $Q=66.33$, $p<0.000$, **Figure 1**). The frequency of rs3129882 allele-G in the Caucasian population was confirmed to be significantly lower than that in the Chinese population (0.448 vs 0.661, $p<0.000$). Subgroup analysis revealed a lack of association of rs3129882 allele-G in the Chinese (OR=0.98, 95% CI: 0.88, 1.09, $I^2=63.2\%$) and Caucasian populations (OR=1.09, 95% CI: 0.99, 1.2, $I^2=83\%$). However, subgroup analysis based on the sequencing technique used showed a significantly increased association of rs3129882 allele-G with PD in the GWAS group (OR=1.21, 95% CI: 1.09, 1.34, $I^2=75.3\%$) but not in the non-GWAS group (OR=1.00, 95% CI: 0.95, 1.07, $I^2=42.1\%$, **Figures 1, 2**). The second subgroup analysis of the Caucasian population further confirmed the association in the GWAS group (OR=1.21, 95% CI: 1.09, 1.34) and not in the non-GWAS group (OR=1.00, 95% CI: 0.95, 1.07, $I^2=0\%$). In the Chinese population, a non-significant association was detected only for the non-GWAS group (OR=1.00, 95% CI: 0.94, 1.06, $I^2=63.2\%$). The meta-regression model, which accounted for 58.3% of the variance, revealed that the results might have been influenced by the DNA sequencing technique used (SE=0.096, 95%

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Table 3. Summary of the reported studies on rs3129882 in Parkinson's disease

No.	Reference	Country	Ethnicity	Sequencing	Allele frequency (G/A)	
					Sporadic PD	Control
1	Mata I F (2011)	Spain	Caucasian	TaqMan	1242/1648	976/1346
2	Pankratz N (2012)	America, Europe	Caucasian	GWAS	4986/3670	4479/3999
3	Ran C (2013)	Sweden	Caucasian	PYRO	378/560	521/751
4	Pihlstrom L (2013)	Norway, Sweden	Caucasian	TaqMan	1023/1525	928/1412
5	Soto-Ortolaza AI (2013), Puschmann A (2011)	America, Ireland, Poland	Caucasian	Direct sequencing	1160/1602	1134/1522
6	Wisseemann WT (2013), Hamza TH (2010), Hill-Burns EM (2011, 2014)	America, Europe	Caucasian	GWAS	1471/1659	1588/2384
7	Simón-Sánchez J (2011)	The Netherlands	Caucasian	GWAS	658/886	1660/2388
8	Guo Y (2011)	China	Mongolian	Direct sequencing	364/204	366/150
9	Chiang HL (2012)	China	Mongolian	PCR-RFLP	1060/584	1038/542
10	Zhao Y (2013)	China	Mongolian	PCR	868/406	877/473
11	Lin CH (2013)	China	Mongolian	TaqMan	591/305	570/334
12	Zhou LL (2014)	China	Mongolian	PCR	430/216	497/193
13	Guo JF (2015)	China	Mongolian	PCR	1305/817	1308/824
14	This study	China (Guangdong)	Mongolian	Direct sequencing	724/360	878/470

Fourteen studies were included in the systemic review. Abbreviations: GWAS, genome-wide association study; PYRO, pyrosequencing; PCR, polymerase chain reaction; PCR-RFLP, restriction fragment length polymorphism analysis of PCR products.

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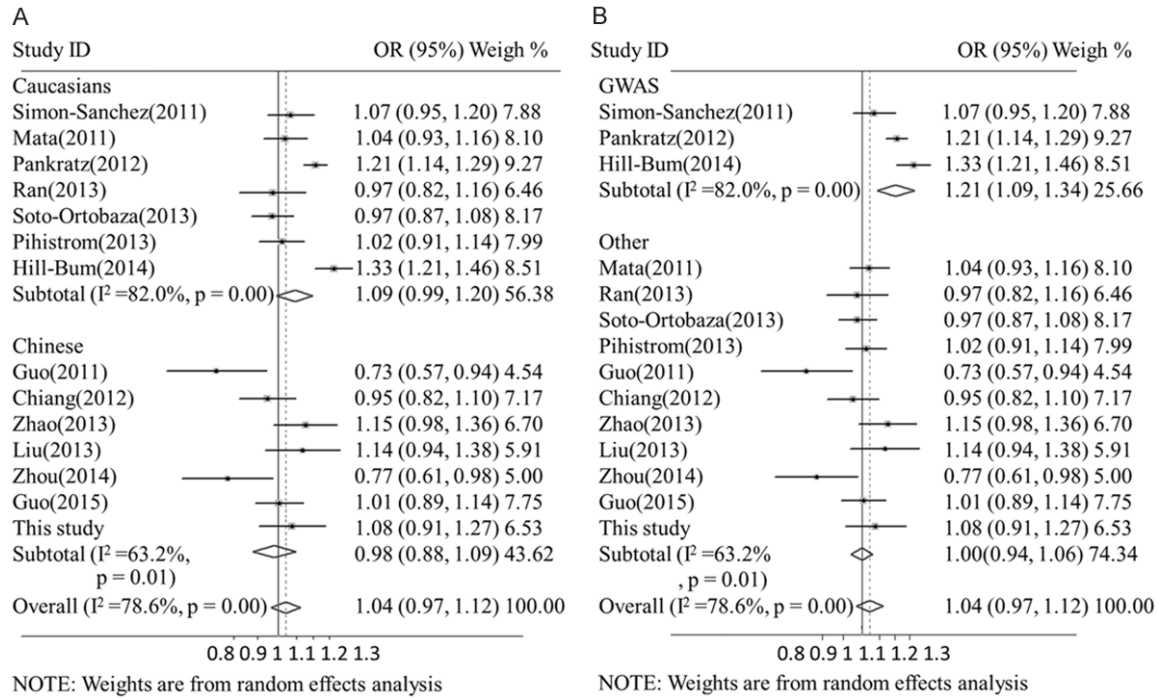


Figure 1. Meta-analysis of the association of rs3129882 in HLA-DRA with Parkinson's disease. The rs3129882 allele-G frequency in the PD population was determined, and sub-group analysis was performed according to ethnicity (A) and the sequencing technique used (B). CI: confidence interval, OR: odds ratio.

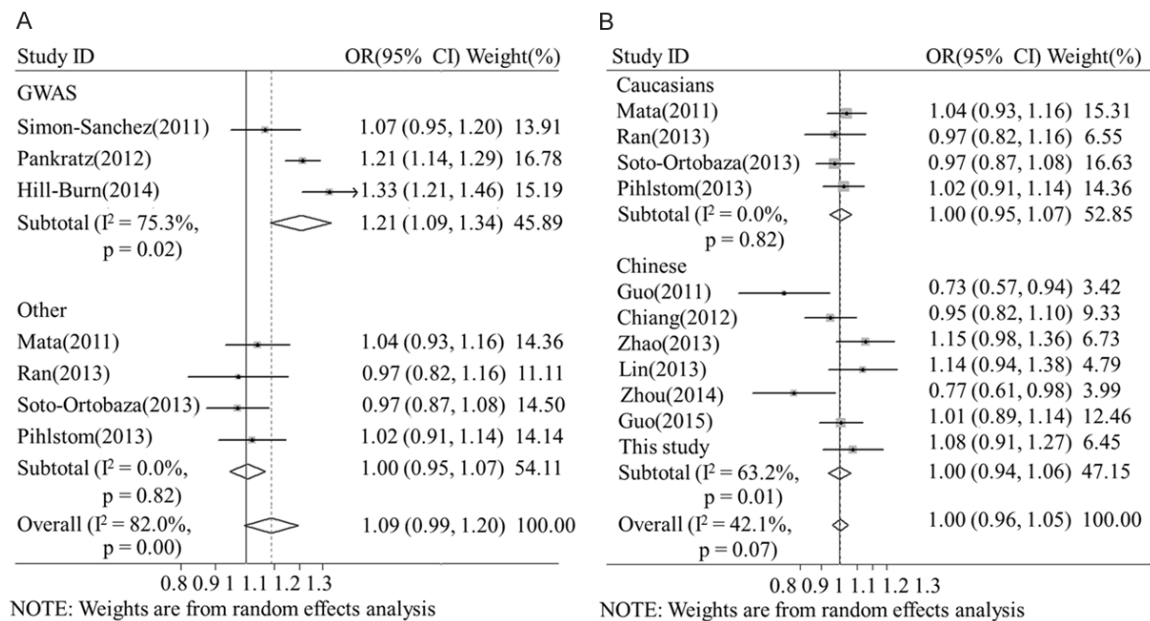


Figure 2. Sub-meta-analysis of rs3129882 in HLA-DRA in Parkinson's disease. The studies of the Caucasian population were collected, and sub-group analysis was performed according to the sequencing technique used (A). Non-GWAS studies were collected, and sub-group analysis was performed according to ethnicity (B). CI: confidence interval, OR: odds ratio.

CI: 0.013, 0.179, $p<0.027$) but not by ethnicity (SE=0.008, 95% CI: -0.064, 0.080, $p<0.808$).

In the Caucasian population, the regression model accounted for 71.2% of the variance and

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Table 4. Regression analysis of rs3129882 in Parkinson's disease

Regression analysis	Co-effort	SE	95% CI	t	P
All (14 studies)					
Methods	0.096	0.038	0.013-0.179	2.55	0.027*
Ethnicity	0.008	0.033	-0.064-0.080	0.25	0.808
Cons	-0.006	0.019	-0.047-0.035	-0.33	0.751
Adjusted R ² =58.3%					
Caucasians (7 studies)					
Methods	0.096	0.033	0.011-0.181	2.91	0.033*
Cons	0.002	0.024	-0.059-0.064	0.09	0.930
Adjusted R ² =71.2%					
Other techniques (11 studies)					
Ethics	0.007	0.030	-0.061-0.076	0.25	0.812
Cons	-0.005	0.017	-0.044-0.034	-0.30	0.768
Adjusted R ² =-35.4%					

The two covariates examined included the method used (categorical variables: GWAS and other techniques) and ethnicity (categorical variables: Chinese and Caucasian), which were analyzed using the random-effects meta-regression model including 14 studies. In sub-group analysis, 7/14 studies of the Caucasians population were used to evaluate the contribution of the method used to the heterogeneity, and 11/14 studies using techniques other than GWAS were applied to evaluate contribution of ethnicity. The co-effort represents the log odds ratios of the covariates, with 95% CIs applied to evaluate the impacts of the covariates. *P<0.05. Abbreviations: Co-effort, regression coefficient; SE, standard error; 95% CI, 95% confidence interval; t, t-value, ratio of log relative risk to standard error; Cons, constant effect; R²=1-SSE/SST, the proportion of variance explained by the covariates.

confirmed that the sequencing technique used significantly contributed to the variance (SE=0.033, 95% CI: 0.011, 0.181, p<0.033). In the non-GWAS studies, the regression model accounted for 35.4% of the variance and confirmed that ethnicity did not significantly contribute to the variance (SE=0.030, 95% CI: -0.061, 0.076, p<0.812) (**Table 4**). Begg's test showed no publication bias in the overall studies, and sensitivity analyses were performed, showing high sensitivity or sensitivity analyses were performed, showing consistent ([Supplemental Datas 3, 4, 5, 6 and 7, 8](#)).

Discussion

The HLA region in chromosome 6p21.3, designated as PARK18, is closely associated with PD [3]. HLA-DRA, an HLA class II gene, encodes a protein that is an MHC class II alpha chain paralog [11]. Earlier studies have shown that MHC class II antigen is increased in microglia in the MPTP model [5], promoting the α -synuclein-induced activation of microglia and resulting in DA neurodegeneration [12]. SNPs in HLA-DRA have been reported to be associated with PD, and rs3129882 is one such candidate SNP

[13, 24, 32]. In this study, we detected 15 SNPs in HLA-DRA by the screening of 1000 kb surrounding rs3129882 in 542 sporadic Chinese PD patients and 674 healthy controls. Our results did not show that any SNP or haplotype was a risk factor for PD in the Chinese population, although the SNP rs75855844 located between DRB5 and DRA and the SNPs rs2395163 and rs424-8166 located between BTNL2 and DRA have been reported to be closely linked to PD in Caucasians [24, 34].

The SNP rs3129882, which is located in the first intron of HLA-DRA [28], has been reported to be involved in inflammation associated with

dopamine neuron degeneration by acting as a regulatory *cis*-element to modify the expression of HLA-DRA, HLA-DQA2 and HLA-DRAB5 [11]. However, the relationship between rs3129882 and PD remains controversial. rs3129882 allele-G has been shown to be a risk factor for sporadic PD in Caucasian populations from America and Europe [13, 24]. In addition, Guo and Zhou have reported that rs3129882 allele-A increases the risk of PD in Han Chinese populations from mainland China [10, 37]. Other studies have demonstrated no association between rs3129882 and PD in populations from America, Australia, the Netherlands, Spain, Japan and China [6, 24, 28, 31, 32, 36]. To clarify the relationship of rs3129882 with PD in the Chinese population, we performed DNA sequencing on 542 PD patients and found no association between rs3129882 and sporadic PD in the allele, dominant, recessive or co-dominant model. A subsequent meta-analysis of 16,123 PD patients and 18,711 controls confirmed our conclusions. Considering our findings and those of other studies, we speculate that the non-significant results of the meta-analysis may have been affected by the highly polymorphic character of

the HLA gene, ethnic-specific effects and environmental factors, as well as the different DNA analysis methods used.

A high level of heterogeneity has been reported in meta-analysis of HLA rs3129882 [38]. The high heterogeneity of most pooled outcomes would cause results to be inconclusive and decrease the strength of evidence [23]. Examination of heterogeneity in rs3129882 studies, which has not been examined previously, may help to identify factors confounding the results [15, 24, 38]. Thus, we performed sub-group analysis and meta-regression to find the sources of heterogeneity. The Eastern and Western populations have been demonstrated to carry different genetic variants for PD risk [25], such as the SNPs in LRRK2 [30]. With regard to rs3129882, we found that its distribution was highly variable among the different ethnicities in HapMap database and may have caused heterogeneity in our meta-analysis. Thus, we conducted sub-group analysis of the Chinese and Caucasian populations to re-evaluate the influence of ethnicity. The results showed that over-estimation of the genetic differences made a tiny contribution to the heterogeneity detected by sub-group and meta-regression analyses. Other factors, such as the sequencing technique used, were taken into account. GWAS is known to be a more powerful tool for detecting genetic risk while minimizing false data compared to other sequencing technologies [21]. Thus, we divided the studies into a GWAS group and a non-GWAS group, in which the studies were conducted using PCR, TaqMan or pyrosequencing techniques. Subgroup analysis revealed a significant association between rs3129882 and PD in the GWAS group. Furthermore, the meta-regression model, which accounted for 58.3% of the variance, confirmed that the sequencing technique used was the main factor contributing to the heterogeneity. Regression analysis of the Caucasian population, excluding the influence of race, showed that the sequencing technique used contributed to 71.2% of the heterogeneity. Taken together, our results suggest that the sequencing technique used is a key factor influencing the sequencing results of rs3129882; therefore, the results regarding the association of rs3129882 with PD may need to be confirmed by GWAS.

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Disclosure of conflict of interest

None.

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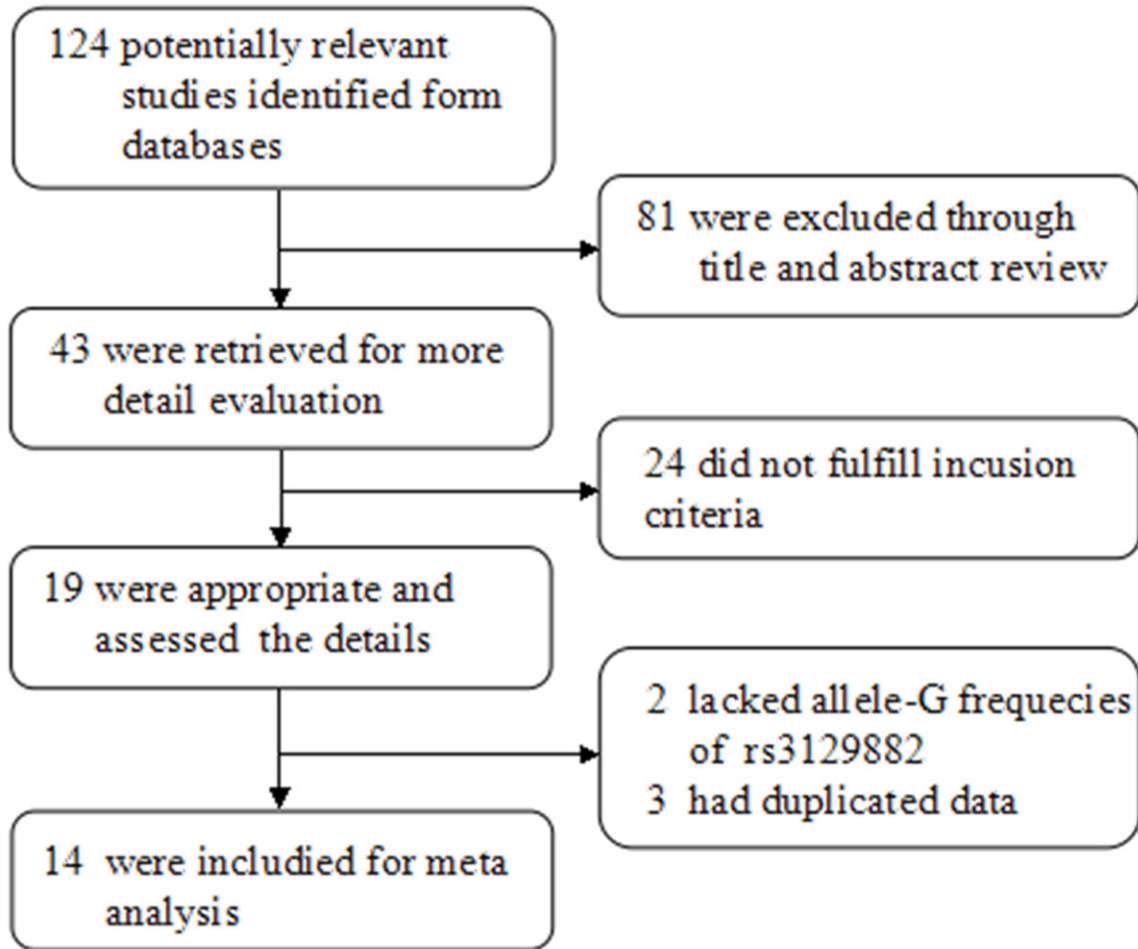
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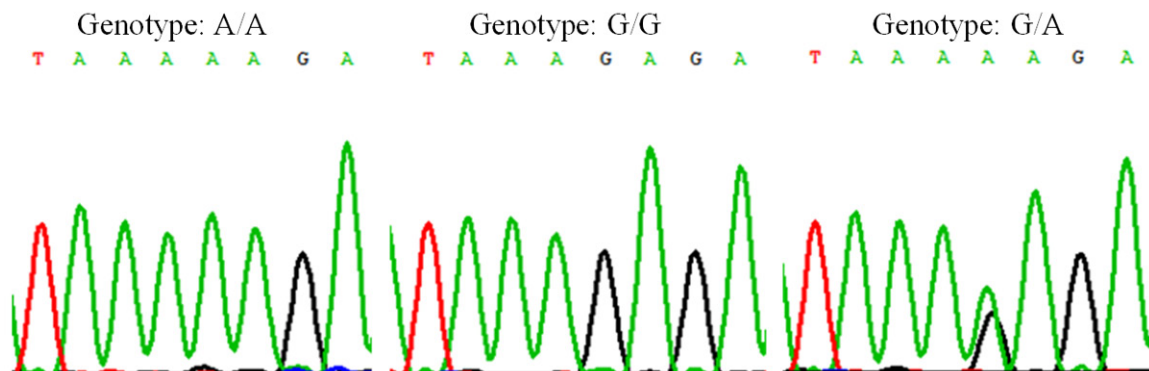
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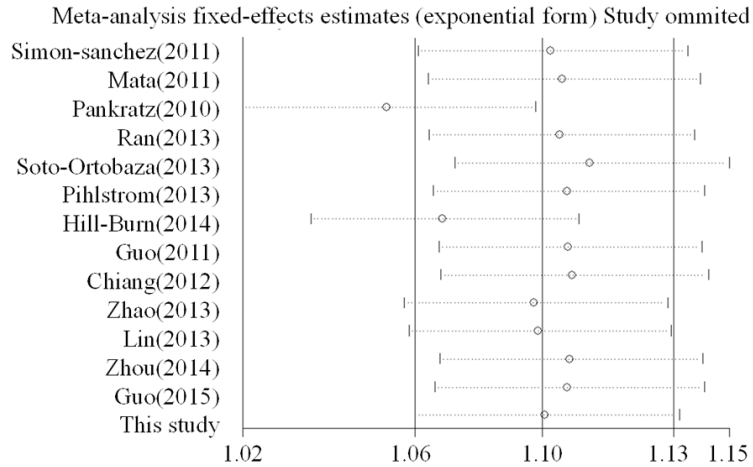


Supplemental Data 1. The procedures of study selection for meta-analysis.

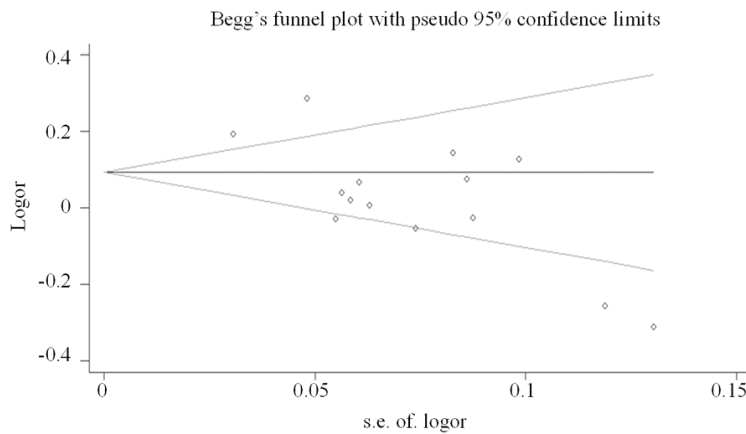


Supplemental Data 2. The genotypic analysis of rs3129882 at HLA-DRA in Chinese population. The direct DNA sequencing analysis showed that rs3129882 at HLA-DRA is heterozygous in PD and healthy control. The rs3129882 genotypes contains A/A, G/G and G/A.

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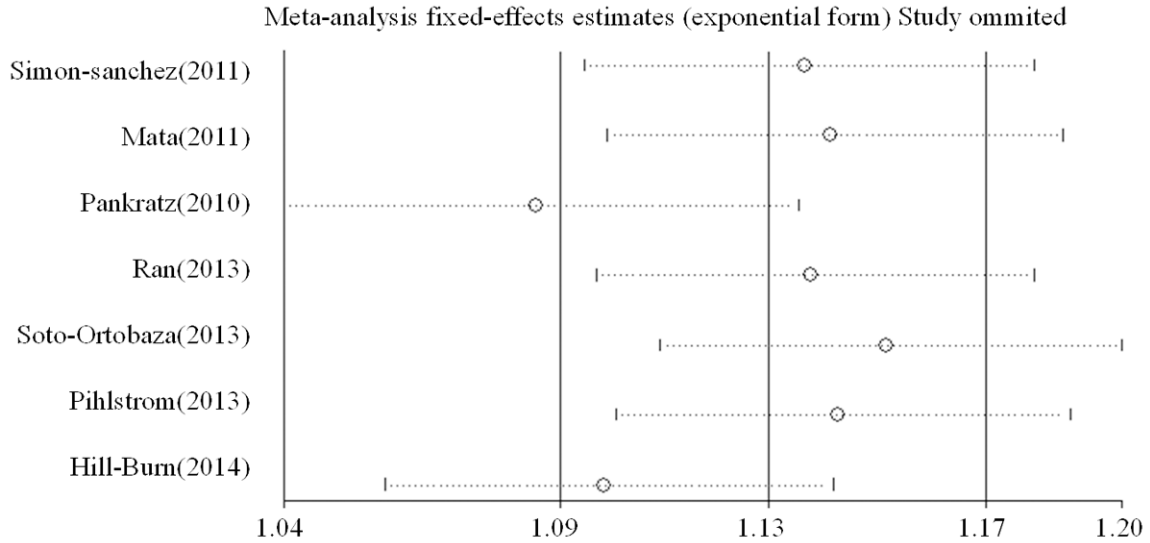


Supplemental Data 3. Sensitivity analysis of the association between rs3129882 and PD in Chinese and Caucasians populations. The omitted studies were listed in the vertical axis and their OR values were shown in horizontal axis. Each plot was draw according to the pooled OR without the omitted studies. The 95% confidence interval was represented by the two ends of every broken line.

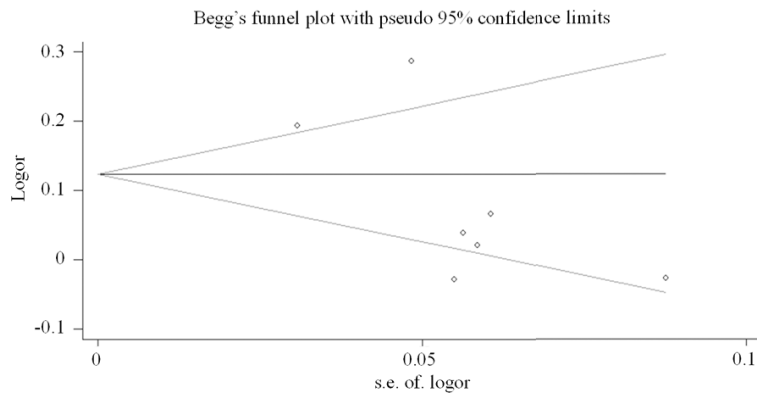


Supplemental Data 4. Begg's funnel plots of the association between rs3129882 and PD in Chinese and Caucasians populations. Each plot was draw according to the OR and s.e. of. logor values of the represented study. The OR means the odds of having the rs3129882 allele G for individuals with PD in relation to the odds for individuals without PD. The represented studies were listed in **Table 3** and the meta-result was in **Figure 1**. S.e. of. logor, natural logarithm of OR; Horizontal line stand for the magnitude of effect.

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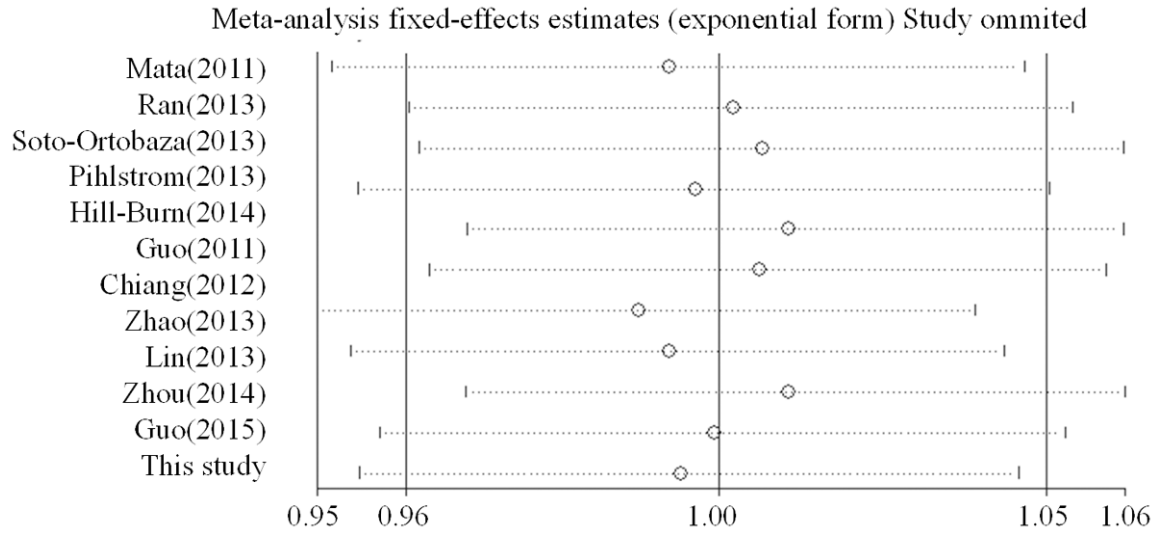


Supplemental Data 5. Sensitivity analysis of the sub-meta-analysis in Caucasians population. The omitted studies were listed in the vertical axis and their OR values were shown in horizontal axis. Each plot was draw according to the pooled OR without the omitted studies. The 95% confidence interval was represented by the two ends of every broken line.

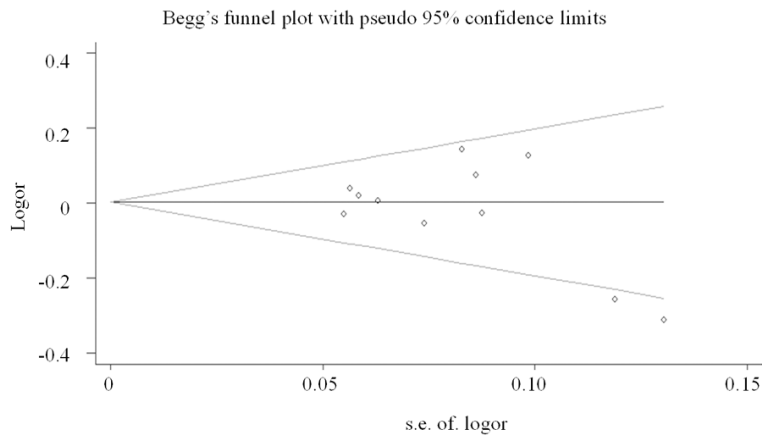


Supplemental Data 6. Begg's funnel plots of sub-meta-analysis in Caucasians population. Each plot was draw according to the OR and s.e. of. logor values and represented one study. The OR means the odds of having the rs3129882 allele G for individuals with PD in relation to the odds for individuals without PD. The represented studies were listed in **Table 3** and the meta-result was in **Figure 1**. S.e. of. logor, natural logarithm of OR; Horizontal line stand for the magnitude of effect.

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Supplemental Data 7. Sensitivity analysis of sub-meta-analysis in non-GWAS studies. The omitted studies were listed in the vertical axis and their OR values were shown in horizontal axis. Each plot was draw according to the pooled OR without the omitted studies. The 95% confidence interval was represented by the two ends of every broken line.



Supplemental Data 8. Begg's funnel plots of sub-meta-analysis in non-GWAS studies. Each plot was draw according to the OR and s.e. of. logor values and represented one study. The OR means the odds of having the rs3129882 allele G for individuals with PD in relation to the odds for individuals without PD. The represented studies were listed in **Table 3** and the meta-result was in **Figure 2**. S.e. of. logor, natural logarithm of OR; Horizontal line stand for the magnitude of effect.