

HLA-DRB1 Alleles Are Associated with the Susceptibility to Sporadic Parkinson's Disease in Chinese Han Population

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Abstract

Immune disorders may play an important role in the pathogenesis of Parkinson's disease (PD). Recently, polymorphisms in the HLA-DR region have been found to be associated with sporadic PD in European ancestry populations. However, polymorphisms in the HLA complex are highly variable with ethnic and geographic origin. To explore the relationships between polymorphisms of the HLA-DR region and sporadic PD in Chinese Han population, we genotyped 567 sporadic PD patients and 746 healthy controls in two independent series for the HLA-DRB1 locus with Polymerase chain reaction-sequence based typing (PCR-SBT). The χ^2 test was used to evaluate the distribution of allele frequencies between the patients and healthy controls. The impact of HLA-DRB1 alleles on PD risk was estimated by unconditional logistic regression. We found a significant higher frequency of HLA-DRB1*0301 in sporadic PD patients than in healthy controls and a positive association, which was independent of onset age, between HLA-DRB1*0301 and PD risk. Conversely, a lower frequency of HLA-DRB1*0406 was found in sporadic PD patients than in healthy controls, with a negative association between HLA-DRB1*0406 and PD risk. Furthermore, a meta-analysis involving 195205 individuals was conducted to summarize the frequencies of these two alleles in populations from various ethnic regions, we found a higher frequency of HLA-DRB1*0301, but a lower frequency of HLA-DRB1*0406 in European ancestry populations than that in Asians, this was consistent with the higher prevalence of sporadic PD in European ancestry populations. Based on these results, we speculate that HLA-DRB1 alleles are associated with the susceptibility to sporadic PD in Chinese Han population, among them HLA-DRB1*0301 is a risk allele while the effect of HLA-DRB1*0406 deserves debate.

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Introduction

Parkinson's disease (PD), the second most common human neurodegenerative disease, is characterized by progressive degeneration of dopaminergic neurons in the substantia nigra (SN) and other brainstem nuclei [1]. To date, the exact etiology of sporadic PD still remains unknown. Immune dysfunctions have been confirmed to be one of the causes of this disease [2–4]. In the brain of PD patients, large numbers of microglia expressing human leucocyte antigen (HLA)-DR have been detected in the SN, particularly in areas of maximal neurodegeneration [5]. The HLA-DR antigen, encoded by HLA-DRA and HLA-DRB alleles, belongs to HLA class II molecules and acts as an antigen-presenting

molecule or regulatory molecule involved in the specific immune response and innate immune response [6]. The highly polymorphic HLA complex plays an important role in genetic susceptibility to human diseases, and different alleles of HLA loci are responsible to variable immune responses among individuals [7].

Associations between genotype of HLA loci and degenerative diseases in the central nerve system (CNS) have been found in many populations. For example, the association between HLA-A*01, A*2402, HLA-DRB1*03 or DRB1*1501 and high risk for Alzheimer disease has been reported [8–11]. Possible associations of HLA-A*2 and A*28, HLA-B*17 and B*18, and HLA-DQB1*06 with PD have also been reported [12–15]. However, in those studies, the enrolled subjects were relatively small by low-

Table 1. Basic information of patients with sporadic Parkinson's disease (PD) and healthy controls.

Sample size	Series1		Series2		Combination	
	PD	Control	PD	Control	PD	Control
Total	374	503	193	243	567	746
Onset age≤50 (Male/Female)	74(31/43)	113(76/37)	33(11/22)	58(20/38)	107(42/65)	171(96/75)
Onset age>50 (Male/Female)	300(177/123)	390(240/150)	160(66/94)	185(75/110)	460(243/217)	575(315/260)

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resolution genotyping analysis, and therefore, the conclusions could be ambiguous. Recently, Saiki *et al.* found that HLA-DRB1*03 was more common among British PD patients than healthy controls with low-resolution typing [16]. In addition, large-scale genome-wide association studies indicated that genetic susceptibility loci for late-onset sporadic PD might have existed in the HLA-DR region, such as rs3129882 in HLA-DRA and chr6:32588205 in HLA-DRB5 [17–19]. These studies suggest that the HLA-DR region is susceptible to the development of PD in European ancestry populations.

However, the HLA complex is highly polymorphic with different ethnic and geographic origins, PD genome-wide association studies (GWAS) study in Japanese shown no evidence of HLA association [20], while the relationship between HLA-DR polymorphism and PD in Chinese Han population has not yet been extensively investigated. Because HLA-DRB1 is the most polymorphic locus in the HLA-DR region [21], we systematically analyzed the polymorphism of HLA-DRB1 alleles among sporadic PD patients and healthy controls from a Han population living in the Guangdong province of the People's Republic of China (PRC) through polymerase chain reaction-sequencing based typing (PCR-SBT). The potential association of HLA-DRB1 alleles with PD in this population was evaluated, and gender and the onset age were taken into account.

Materials and Methods

Patients

A total of 567 sporadic PD patients and 746 unrelated healthy controls of Han population root from Guangdong province of the PRC were enrolled for this study (Basic information shown in Table 1). Each subject signed an informed consent before participating. The Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University approved the protocol for this study. All PD patients were diagnosed according to the modified UK Brain Bank criteria [22] at the Parkinson Clinic Center in the First Affiliated Hospital of Sun Yat-sen University from 2007 to 2012. The healthy controls were randomly selected from blood donor volunteers in the health examination center of The First Affiliated Hospital of Sun Yat-sen University. The average age of the PD patients and healthy controls was 62.20 ± 19.10 years (range: 16–88) and 58.52 ± 14.30 years (range: 18–85) respectively.

DNA Extraction and HLA Typing

Whole blood samples were collected from PD patients and healthy controls. Genomic DNA was extracted from 4 ml of whole blood using the QIA-amp DNA Mini Kit (QIAGEN, German) according to the manufacturer's protocols. Extracted genomic DNA samples were stored at -80°C until genotyping was performed. The primer pairs recommended by the International

Histocompatibility Working Group (IHWG) were used to subtype the alleles of HLA-DRB1. HLA-DRB1 allele groups were typed by polymerase chain reaction-sequencing based typing (PCR-SBT) described previously [23]. Briefly, 22 group-specific primer pairs were used to amplify exon 2 of HLA-DRB1 using a thermal cycler (model 9700, Applied Biosystems, Foster City, CA, USA). PCR products were purified by incubating them with exonuclease at 37°C for 30 min and then with shrimp alkaline phosphatase (SAP) at 80°C for 15 min. We sequenced each purified PCR product using the ABI BigDye Terminator3.1 Cycle Sequencing Kit (Applied Biosystems, USA) and the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). The sequence data were analyzed using the Match Tools and Navigator software (Match Tools Allele Identification package, Applied Biosystems, USA).

Statistic Analyses

Alleles were tested for deviation from Hardy-Weinberg equilibrium (HWE) using the SHEsis software platform [24]. Phenotype Frequency was defined as the total number of carriers in the population (Carriers/n), Allele Frequency was defined as the total number of copies of the allele in the population sample (Alleles/2n). The χ^2 test was used to evaluate the distribution of allele frequencies between the patients and healthy controls, The Fisher exact test was used when expected frequencies were less than five. In addition, Bonferroni adjusted *p* values (*pc*) were used to avoid an alpha inflation when chaining statistical tests, the number of chaining statistical tests was defined as the number of the detected alleles. The association between PD and alleles was represented by odds ratio (OR), which was estimated by unconditional logistic models adjusted for the age of onset and gender. Statistical power of every allele can be calculated by the

$$\text{formula } "Z_{\beta} = \frac{|\pi_1 - \pi_2| - Z_{\alpha} \sqrt{\pi(1-\pi) \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}}{\sqrt{\pi_1(1-\pi_1)/n_1 + \pi_2(1-\pi_2)/n_2}}, \text{ Power} = 1 - \beta "$$

π_1 = the phenotype frequency of the allele in group1, π_2 = the phenotype frequency of the allele in group2, n_1 = the sample size of group1, n_2 = the sample size of group2, $\pi = (n_1 * \pi_1 + n_2 * \pi_2) / (n_1 + n_2)$, $\alpha = 0.05$, $Z_{\alpha/2} = 1.960$ (two sides). Calculations were carried out using SPSS-13.0 software (SPSS Inc., Chicago, IL). A *pc* < 0.05, statistic Power > 0.75 was regarded as statistically significant.

Results

The Distribution of HLA-DRB1 Alleles between PD Patients and Healthy Controls

HLA-DRB1 genotype frequencies in both PD patients and healthy controls in two series were remained constant according to

Table 2. Frequencies of HLA-DRB1 phenotypes and alleles in patients with Parkinson's disease (PD) and healthy controls.

HLA-DRB1 Alleles	Series 1			Series 2			Combination P/Pc
	PD N = 374	Control N = 503	P/Pc	PD N = 193	Control N = 243	P/Pc	
0101	4(0.0053)	12(0.0119)	ns	–	4(0.0082)	ns	
0102	–	2(0.0020)	ns	–	–	ns	
0301	63(0.0869)	48(0.0477)	0.001/0.041	27(0.0699)	18(0.0391)	0.025/ns	9.728E-5/4.572E-3
0304	–	1(0.0010)	ns	–	–	ns	
0401	3(0.0040)	10(0.0099)	ns	2(0.0052)	7(0.0144)	ns	
0402	–	1(0.0010)	ns	–	–	ns	
0403	23(0.0307)	16(0.0159)	0.035/ns	15(0.0389)	9(0.0185)	ns	0.005/ns
0404	14(0.0187)	11(0.0109)	ns	6(0.0155)	3(0.0062)	ns	
0405	38(0.0508)	47(0.0467)	ns	18(0.0466)	26(0.0535)	ns	
0406	1(0.0013)	30(0.0308)	8.036E-7/3.295E-5	3(0.0078)	12(0.0267)	0.097/ns	3.229E-6/1.518E-4
0407	–	1(0.0010)	ns	–	–	ns	
0410	–	1(0.0010)	ns	–	1(0.0021)	ns	
0701	36(0.0495)	53(0.0567)	ns	19(0.0570)	38(0.0823)	ns	
0702	–	2(0.0020)	ns	–	–	ns	
0801	–	2(0.002)	ns	–	–	ns	
0802	3(0.0040)	5(0.0050)	ns	–	1(0.0021)	ns	
0803	53(0.0749)	70(0.0716)	ns	31(0.0881)	36(0.0802)	ns	
0809	1(0.0013)	5(0.0050)	ns	–	–	ns	
0901	115(0.1618)	159(0.167)	ns	57(0.1580)	84(0.1831)	ns	
1001	7(0.0094)	16(0.0159)	ns	3(0.0078)	7(0.0144)	ns	
1101	34(0.0455)	50(0.0507)	ns	19(0.0492)	21(0.0432)	ns	
1104	–	2(0.0020)	ns	1(0.0026)	–	ns	
1106	2(0.0027)	–	ns	–	–	ns	
1201	25(0.0334)	46(0.0497)	ns	10(0.0259)	18(0.0370)	ns	
1202	80(0.1123)	81(0.0875)	0.045/ns	33(0.0907)	41(0.0885)	ns	ns
1218	3(0.0040)	–	ns	–	–	ns	
1220	–	–	ns	–	1(0.0021)	ns	
1301	2(0.0027)	8(0.0080)	ns	4(0.0104)	2(0.0041)	ns	
1302	17(0.0227)	34(0.0348)	ns	10(0.0259)	16(0.0329)	ns	
1305	–	1(0.0010)	ns	–	–	ns	
1312	14(0.0187)	8(0.0080)	0.044/ns	7(0.0181)	5(0.0103)	ns	0.027/ns
1403	3(0.0040)	4(0.0040)	ns	1(0.0026)	3(0.0062)	ns	
1404	4(0.0053)	8(0.0080)	ns	3(0.0078)	3(0.0062)	ns	
1405	21(0.0281)	19(0.0189)	ns	12(0.0311)	10(0.0206)	ns	
1406	–	–	ns	–	1(0.0021)	ns	
1407	–	2(0.0020)	ns	–	2(0.0041)	ns	
1410	–	–	ns	–	1(0.0021)	ns	
1418	–	4(0.0040)	ns	–	–	ns	
1422	–	1(0.0010)	ns	–	–	ns	
1454	28(0.0428)	21(0.0209)	0.035/ns	15(0.0389)	13(0.0288)	ns	0.021/ns
1501	75(0.1016)	111(0.1183)	ns	42(0.1192)	50(0.1111)	ns	
1502	27(0.0361)	33(0.0328)	ns	12(0.0311)	13(0.0267)	ns	
1504	–	1(0.0010)	ns	1(0.0026)	–	ns	
1545	–	–	ns	–	1(0.0021)	ns	
1601	–	–	ns	–	1(0.0021)	ns	
1602	30(0.0414)	41(0.0427)	ns	19(0.0492)	19(0.0391)	ns	

Phenotype Frequency (Allele Frequency) was presented in every cell. “–”: the allele was not been detected. Phenotype Frequency: Percentage of individuals who have the allele (Individuals/N) in percentage format. Allele Frequency: Total number of copies of the allele in the population sample (Alleles/2N) in decimal format, Pc = correction of P value (Bonferroni adjustment), Pc < 0.05 is considered as significant, ns = not significant. Patients had significant higher frequencies of HLA-DRB1*0301 and lower frequency of HLA-DRB1*0406 than healthy controls did.

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HWE. A total of 46 HLA-DRB1 alleles were detected in all studied individuals, among them 41 alleles were detected in series 1, 34 alleles were detected in series 2.

We found that PD patients presented higher allele frequencies of HLA-DRB1*0301 (0.0869 vs. 0.0477, $p=0.001$ in series 1, 0.0699 vs. 0.0391, $p=0.025$ in series 2, 0.0811 vs. 0.0449, $p=9.728E-5$ in combined series 1 and series 2), DRB1*0403 (0.0307 vs. 0.0159, $p=0.035$ in series 1), DRB1*1202 (0.1123 vs. 0.0875, $p=0.045$ in series 1), DRB1*1312 (0.0187 vs. 0.0080, $p=0.044$ in series 1), and DRB1*1454 (0.0428 vs. 0.0209, $p=0.035$ in series 1) than the healthy controls. Conversely, the HLA-DRB1*0406 was found with lower allele frequency in PD patients compared to healthy controls (0.0013 vs. 0.0308, $p=8.036E-7$ in series 1, 0.0078 vs. 0.0267, $p=0.097$ in series 2, 0.0035 vs. 0.0295, $p=3.229E-6$ in combined series 1 and series 2). However, after Bonferroni adjustment only the differences of distribution of the HLA-DRB1*0301 ($p_c=0.041$ in series 1, $p_c=4.572E-3$ in combined series 1 and series 2) and HLA-DRB1*0406 ($p_c=3.295E-5$ in series 1, $p_c=1.518E-4$ in combined series 1 and series 2) alleles were statistically significant (statistic power >0.75) (Table 2). Using unconditional logistic regression analysis adjusted for the age of onset and gender, we found that the HLA-DRB1*0301 allele was positively associated with the risk of PD (OR = 1.963, $p=0.001$ in series 1, OR = 2.135, $p=0.019$ in series 2, OR = 2.048, $p=3.952E-5$ in combined series 1 and series 2), while the HLA-DRB1*0406 allele might be negatively associated with the risk of PD (OR = 0.043, $p=0.002$ in series 1, OR = 0.312, $p=0.077$ in series 2, OR = 0.118, $p=5.002E-5$ in combined series 1 and series 2).

The Relationship between HLA-DRB1*0301 and PD Risk

To further analyze the relationship between HLA-DRB1*0301 and PD risk, we compared the allele frequencies of HLA-DRB1*0301 between subgroups classified by the gender and the onset age of all subjects through 20 independent χ^2 tests and statistic power calculation described previously. We found that the

allele frequencies of HLA-DRB1*0301 were higher in both PD subgroups (onset age ≤ 50 and onset age >50) than in their healthy control subgroups (0.0678 vs. 0.0263, $p=0.001$, 0.0342 vs. 0.0213, $p=0.008$, respectively), these differences were only occurred in male subgroups (0.0892 vs. 0.0286, $p=0.001$, 0.0412 vs. 0.0230, $p=0.014$, respectively). More interestingly, we found that the allele frequency of HLA-DRB1*0301 was higher in patients with an onset age ≤ 50 than in patients with an onset age >50 (0.0678 vs. 0.0342, $p=0.001$ for all patients, 0.0892 vs. 0.0412, $p=0.003$ for male patients and 0.0538 vs. 0.0265, $p=0.046$ for female patients). After Bonferroni adjustment, the differences of allele frequency between PD subgroups (onset age ≤ 50) and healthy control subgroups (onset age ≤ 50) and PD subgroups (onset age >50) was statistically significant ($P_c=0.020$ and $P_c=0.020$, statistic Power >0.75 , respectively)(Fig.1). Using stratification analyses and unconditional logistic regression analysis adjusted for gender, we found that the positive association between HLA-DRB1*0301 and PD risk was independent of onset age (onset age ≤ 50 : OR = 3.350, $P=4.039E-4$, onset age >50 : OR = 1.726, $P=0.007$).

The Allele Frequencies of HLA-DRB1*0301 and HLA-DRB1*0406 in Populations from Various Ethnic Regions

To get the allele distribution of HLA-DRB1*0301 and HLA-DRB1*0406 in worldwide populations, we conducted a meta-analysis to summarize the allele frequencies of HLA-DRB1*0301 and HLA-DRB1*0406 in populations from various ethnic regions. A systemic literature review was made to identify 32 studies involving 195205 individuals met criteria for the meta analysis. (Table 3). The inclusion criteria was (i) randomised trial, no selection bias. (ii) subjects were all collected from healthy adult volunteers, and sample size ≥ 500 . (iii) protocols for HLA-DRB1 genotyping: High-resolution genotyping analysis. (iv) confirm to Hardy-Weinberg equilibrium. (v) The allele frequencies were calculated by direct gene counting method. (vi) The allele frequencies of HLA-DRB1*0301 and HLA-DRB1*0406 were

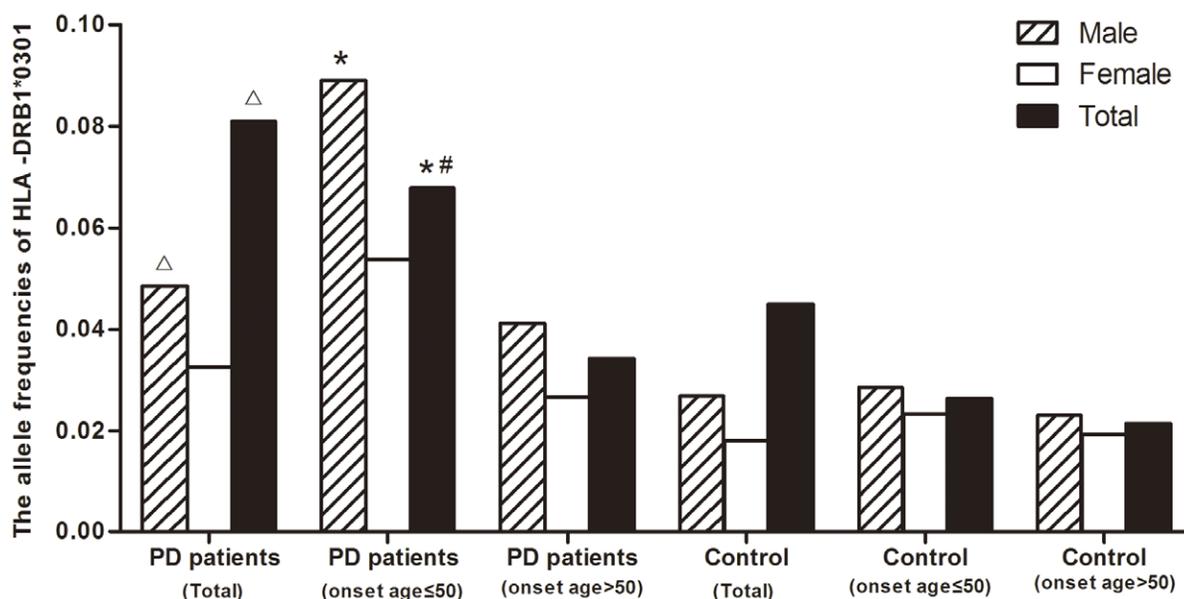


Figure 1. Comparison of the allele frequencies of HLA-DRB1*0301 among various groups. All subjects were classified by gender and age of onset of Parkinson's disease (PD). $\Delta P_c < 0.05$ the allele frequency of HLA-DRB1*0301 in PD patients (total) vs. the one in healthy control (total), $* P_c < 0.05$ the allele frequency of HLA-DRB1*0301 in PD patients (onset age ≤ 50) vs. the one in healthy control subgroup (onset age ≤ 50), $\# P_c < 0.05$ the allele frequency of HLA-DRB1*0301 in PD patients (onset age ≤ 50) vs. the one in PD patients (onset age >50). doi:10.1371/journal.pone.0048594.g001

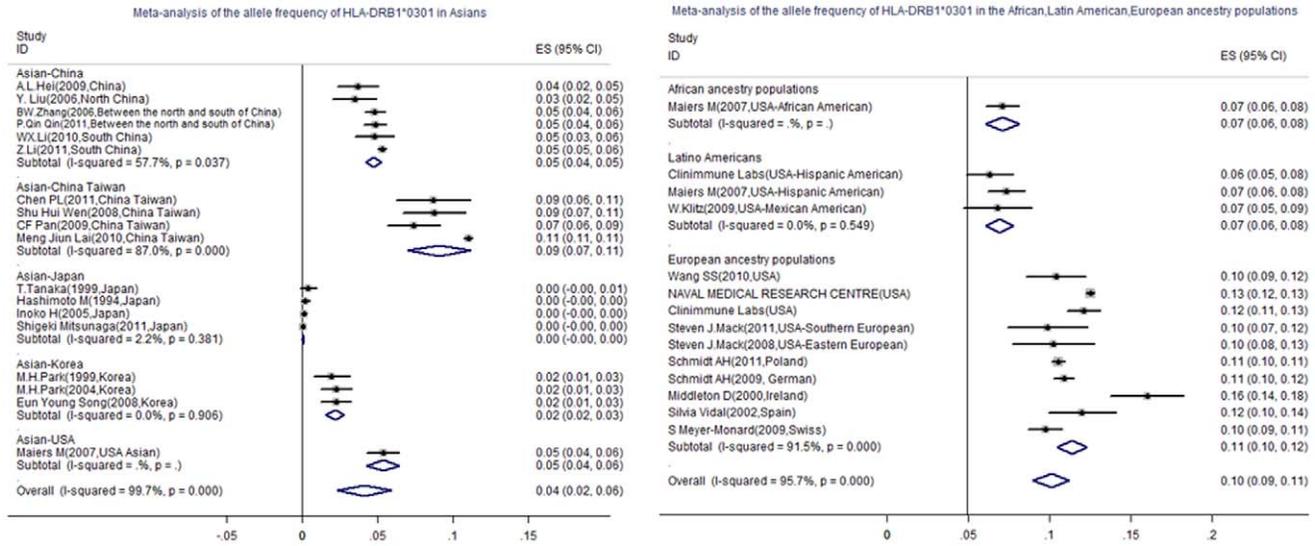


Figure 2. Forest plots summarizing the Allele frequency of HLA-DRB1*0301 in Asian and European ancestry populations from various regions. The allele frequency was indicated as ES (95%CI). The distribution of HLA-DRB1*0301 was inequality in the worldwide populations, European ancestry populations presented a higher allele frequency of HLA-DRB1*0301 than African and Latin American ancestry populations and Asians (0.114 Vs. 0.071, 0.069, 0.041). In intra-Asian, Taiwanese presented a higher allele frequency of HLA-DRB1*0301 than populations in USA, mainland China, and Korea(0.091 Vs.0.054, 0.047, 0.022). However, HLA-DRB1*0301 was a rare allele in Japanese. doi:10.1371/journal.pone.0048594.g002

detected all together. The Excluding criteria was (i) the basic information (eg. race, region) of subjects was unclear. (ii) there was a limitation in subject collections (eg. gender, age, smoker) (iii) the calculation method for allele frequency was unspecified. Calculations were carried out using Stata-11.0 software (StataCorp, College Station, TX, USA). Using a meta-analysis, we found that (i) the distribution of HLA-DRB1*0301 and HLA-DRB1*0406 was inequality in populations from various ethnic regions: European ancestry populations presented a higher allele frequency of HLA-DRB1*0301 but a lower allele frequency of HLA-

DRB1*0406 than Asians (0.114 Vs. 0.041, 0.001 Vs. 0.031, respectively). European ancestry populations presented a higher allele frequency of HLA-DRB1*0301 than African and Latin American ancestry populations (0.114 Vs. 0.071, 0.069), and HLA-DRB1*0406 was presented as a rare allele in these regions. (ii) In intra-Asians: Taiwanese presented a higher allele frequency of HLA-DRB1*0301 than populations from USA, mainland China, and Korea (0.091Vs.0.054, 0.047, 0.022), However, HLA-DRB1*0301 was presented as a rare allele in Japanese. Korean presented a higher allele frequency of HLA-DRB1*0406 than

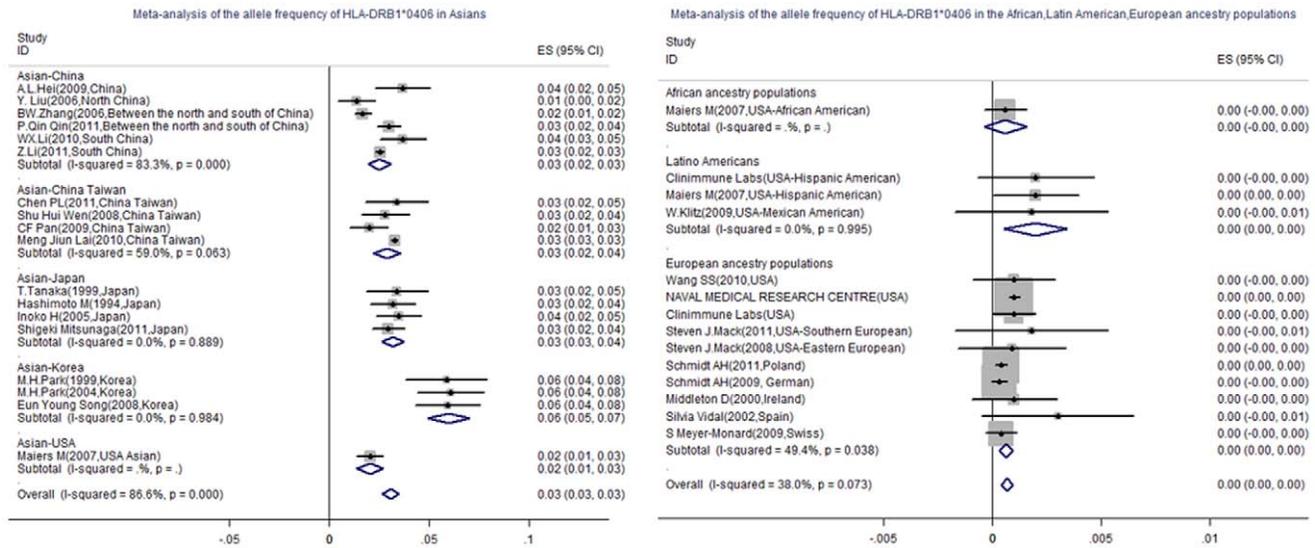


Figure 3. Forest plots summarizing the Allele frequency of HLA-DRB1*0406 in Asian and European ancestry populations from various regions. The allele frequency was indicated as ES (95%CI). The distribution of HLA-DRB1*0406 was inequality in the worldwide populations, it was common in Asians but rare in African, Latin American and European ancestry populations (0.031 Vs.0.001, 0.002, 0.001). In intra-Asian, Korean presented a higher allele frequency of HLA-DRB1*0406 than populations in Japan, China Taiwan, mainland China, and USA(0.060 Vs.0.032,0.029,0.025,0.021). doi:10.1371/journal.pone.0048594.g003

Table 3. The allele frequencies of HLA-DRB1*0301 and HLA-DRB1*0406 in populations from various ethnic regions.

Author	Ethnic origin	Geographic Region	Size	Allele	Frequency
A. L.Hei [25]	Asian	China(Beijing,Suzhou,Shenzhen)	718	0.0369	0.0369
Y. Liu [26]	Asian	North China(Beijing, Tianjin)	618	0.0348	0.0138
BW.Zhang [27]	Asian	^a North China(Henan province)	3874	0.0484	0.0169
P.Qin Qin [28]	Asian	^a Between the north and south	3238	0.0485	0.0298
WX.Li [29]	Asian	^a South China(Hubei province)	1013	0.0479	0.0370
Z.Li [30]	Asian	^a South China(Guangdong)	20621	0.0531	0.0254
Chen PL [31]	Asian	China Taiwan	504	0.0870	0.0340
ShuHui Wen [32]	Asian	^b China Taiwan	710	0.0880	0.0282
CF Pan [33]	Asian	China Taiwan	855	0.0740	0.0200
Meng Jiun	Asian	^b China Taiwan	46682	0.1103	0.0327
T.Tanaka [35]	Asian	^f Japan	525	0.0040	0.0340
Hashimoto	Asian	Japan	916	0.0020	0.0320
Inoko H [37]	Asian	Japan	1018	0.0010	0.0350
Shigeki	Asian	Japan	1616	0.0003	0.0297
M.H.Park [39]	Asian	Korea	510	0.0196	0.0588
M.H.Park [40]	Asian	Korea	800	0.0230	0.0610
Eun Young	Asian	Korea	800	0.0225	0.0594
Maiers M [42]	Asian	USA	1772	0.0537	0.0206
Maiers M [42]	African	USA	2411	0.0707	0.0006
Clinimmune	Hispanic American	^c USA	1058	0.0630	0.0020
Maiers M [42]	Hispanic American	USA	1999	0.0733	0.0020
W.Klitz [43]	Mexican American	USA	553	0.0678	0.0018
Wang SS [44]	European ancestry	USA	1070	0.1039	0.0010
NAVAL MEDICAL	European ancestry	^f USA	61655	0.1250	0.0010
Clinimmune	European ancestry	^c USA	3830	0.1210	0.0010
Steven	Southern European	USA	552	0.0987	0.0018
Steven	Eastern European	USA	558	0.1022	0.0009
Schmidt AH [47]	Eastern European	^d Poland	20653	0.1053	0.0004
Schmidt AH [48]	Western European	German	8862	0.1087	0.0003
Middleton D [49]	Western European	Ireland Northern	1000	0.1600	0.0010
Silvia Vidal [50]	Western European	^e Spain Barcelona	941	0.1200	0.0030
S Meyer	Western European	Swiss	3093	0.0972	0.0004

These data information were collected from the Allele Frequency Net Database [52] except reference 25,27–30,33–34,38–39,41,45,51. Allele Frequency: Total number of copies of the allele in the population sample (Alleles/2n) in decimal format. a: data from Chinese National Marrow Donor Program(CMDP), b: data from Tzu Chi Taiwan Marrow Donor Registry (TCTMDR), c: data from USA Colorado Univ. Cord Blood Bank, d: data from Poland DKMS, e: data from Umbilical Cord Blood Bank of Barcelona, f: data in Allele frequency net was calculated from Phenotype Frequencies assuming Hardy-Weinberg proportions. doi:10.1371/journal.pone.0048594.t003

populations from Japan, China Taiwan, mainland China and USA (0.060 Vs.0.032, 0.029, 0.025, 0.021). (Figure 2&3).

Discussion

In this study, we found that HLA-DRB1 alleles may be contributing to susceptibility to sporadic PD in the Chinese Han population in the Guangdong Province of PRC. The allele frequency of HLA-DRB1*03 was significantly higher in PD patients than in healthy controls (0.0811 vs. 0.0449, $pc = 1.789E-3$, statistic power >0.75), which was identical with the report in European ancestry populations [16]. Furthermore, we found that HLA-DRB1*0301, the most common subtype of HLA-DRB1*03, had a strong association with PD development.

HLA-DRB1*0301 has been reported contribute to the genetic susceptibility of some autoimmune diseases such as type 1 diabetes and multiple sclerosis [53–54]. In our study, the allele frequency of HLA-DRB1*0301 was significantly higher in PD patients than in healthy controls (0.0811 vs. 0.0449, $pc = 4.572E-3$, statistic power >0.75). Furthermore, the allele frequency was significantly higher in patients with an onset age ≤ 50 than in patients with an onset age >50 (0.0678 vs. 0.0342, $pc = 0.012$, statistic Power >0.75), suggesting that people carrying the HLA-DRB1*0301 allele may have a tendency for an early onset of PD. Molecules encoded by polymorphic HLA alleles elicit different T-cell killing effects by presenting different peptide-binding preferences [55]. In pulmonary sarcoidosis, patients carrying the HLA-DRB1*0301 allele have shown more AV2S3 positive T-cells, but less regulatory T-cells than those carrying other HLA-DRB1 alleles [56–57].

Table 4. The tag SNPs of HLA-DRB1 alleles in four HapMap populations.

HLA-DRB1 alleles	CHB	JPT	CEU	YRI
0301	rs5000803	–	rs2040410	rs2040410
	rs3129299	–	rs2187688	rs9277489
	rs7769979	–	–	rs9275229
0401	–	rs1150758	–	rs3129763
0403	rs2075800	rs169494	rs7454108	–
	rs2395175	rs3763349	rs206765	–
	–	–	rs399604	–
0406	–	rs2395175	–	–
0701	rs3129859	rs6936863	rs7745002	–
0802	–	rs3129888	–	–
0803	rs3129859	rs2395148	–	–
0901	rs2395185	rs2187818	–	–
1201	–	–	–	rs4248166
1202	rs6916742	rs4418214	–	–
	rs646984	–	–	–
1301	rs4947342	–	rs2395173	rs3134942
1302	rs11758998	rs6936204	rs4434496	rs2157339
1401	–	–	–	rs1612904
1406	–	rs3129888	–	–
1501	rs7773756	–	–	–
1502	rs2858880	rs3135365	rs2858880	–

Tag SNPs were obtained from the references [75–80]. “–”: the tag SNP has not been detected. rs9275229 has merged into rs2858324. CHB: Han Chinese in Beijing, JPT: Japanese in Tokyo, CEU: Utah Residents with Northern and Western European Ancestry, YRI: Yoruba in Ibadan, Nigeria. doi:10.1371/journal.pone.0048594.t004

Interestingly, several studies reported that regulatory T-cells could mediate neuroprotection through modulation of microglia oxidative stress and inflammation, while profound T-cell responses could exacerbate neuroinflammation and induce dopaminergic neurodegeneration [58–61]. Thus, we speculated that the HLA-DRB1*0301 allele may be related to younger patients through eliciting strong inflammatory responses.

The HLA-DRB1*0406 allele, a subtype of HLA-DRB1*04, whether play a role to prevent the development of sporadic PD deserves debate. The HLA-DRB1*0406 allele is rare in European ancestry populations, but common in Asian ancestry populations [25–52]. Previous studies have shown that it is associated with susceptibility to insulin autoimmune syndrome, silicosis, prostate cancer, and pemphigus in Asian ancestry populations [62–65], which implied that HLA-DRB1*0406 is a risk allele for immune dysfunction diseases. In our study, the allele frequency of HLA-DRB1*0406 in PD patients was significantly lower than in healthy controls (0.0013 vs. 0.0308, $p = 3.295E-5$, statistic Power >0.75) in series 1, but the difference was not achieve statistical significance in series 2 (0.0078 vs. 0.0267, $p = 0.097$, $pc = 1$), this inconsistency may be due to a sampling bias. We noticed that the allele frequencies of HLA-DRB1*0403, another subtype of HLA-DRB1*04, was presented higher in PD patients than in healthy controls in series 1 although the difference was not significant after Bonferroni adjustment (0.0307 vs. 0.0159, $p = 0.035$, $pc = 1$). There was no report that HLA-DRB1*04 have two opposing effects in the disease. Therefore, whether HLA-DRB1*0406 plays

a unique neuroprotective role in the PD development of Chinese Han population still needs to be investigated.

After summarizing the allele frequencies of HLA-DRB1*0301 and HLA-DRB1*0406 in populations from various ethnic regions, we confirmed that the distribution of the two HLA-DRB1 alleles was highly polymorphic with different ethnic and geographic, this phenomenon was more obviously in vast and complicated races regions, such as mainland China, China Taiwan, and America. We also found a higher frequency of HLA-DRB1*0301, but a lower frequency of HLA-DRB1*0406 in European ancestry populations than in Asian ancestry populations. This was consistent with the higher prevalence of PD in European ancestry populations than in Asian ancestry populations [66–69]. However, we could not analyze the correlation between the allele frequencies of these loci and prevalence of sporadic PD because the allele frequency data was incomplete and the prevalence data was collected using different methodologies in the worldwide population.

We noticed that the allele frequencies of HLA-DRB1*1202, DRB1*1312, and DRB1*1454 were also higher in PD patients than in healthy controls. However, the difference was not significant after Bonferroni adjustment, the statistic power in those alleles are at moderate level (0.5 < statistic Power < 0.75). Several studies reported these alleles unequivocally associated with immune-related diseases [70–74]. The possibility cannot be ruled out that these alleles may be either susceptibility genes or linkage disequilibrium with other susceptibility genes contributing to the etiology of PD. In addition, some rare alleles were detected because of random sampling, we lost most of the statistical power in these alleles due to their very small allele frequencies.

Recent studies have shown that some common HLA alleles can be marked with SNP-based tags [75–80]. We listed tag SNPs of the HLA-DRB1 alleles (Table 4), and compared it with the reported genetic susceptibility SNPs in the HLA-DR region to sporadic PD in European ancestry populations. We found that (i) these tag SNPs are all located at a considerable distance from the HLA-DRB1 allele. (ii) tag SNPs are likely to differ between populations, HLA-DRB1*0406 in JPT (Japanese in Tokyo) and HLA-DRB1*0403 in CHB (Han Chinese in Beijing) shared the same tag SNP: rs2395175, our study showed that HLA-DRB1*0403 was more common in PD patients than in controls, but the difference was no statistic significance, that partially explained why PD GWAS study in Japanese saw no evidence of HLA association even though the HLA-DRB1*0406 allele appears more frequent in Japan [20]. (iii) rs3129859 (tag HLA-DRB1*0701 in CHB), rs3129888 (tag HLA-DRB1*0802, -DRB1*1406 in JPT), and rs3763313 (tag HLA-DRB1*0803 in JPT) have been reported in association with the genetic susceptibility to PD in European ancestry populations [17–19], but there was no evidence showed that HLA-DRB1*0701, -DRB1*0802, -DRB1*0803, -DRB1*1406 were susceptibility loci to PD. The inconsistency may be arising from highly ethnic and region differences in HLA allele and SNP frequencies. In addition, reports on map of HLA alleles and its tag SNPs were few.

In conclusion, our study indicated that HLA-DRB1 alleles are associated with sporadic PD in a Chinese Han population, further research will be required to explore the role of HLA-DRB1 alleles in the pathogenesis of Parkinson' disease.

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Author Contributions

Conceived and designed the experiments: CS ZL PX. Performed the experiments: CS LW FL YL LX. Analyzed the data: CS. Contributed reagents/materials/analysis tools: JL FZ PK RX. Wrote the paper: CS PX.

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