

Single nucleotide polymorphisms in the FAM167A-BLK gene are associated with polymyositis/dermatomyositis in the Han Chinese population

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Abstract Idiopathic inflammatory myopathies (IIMs) are autoimmune diseases influenced by genetic background and environmental factors. Recently, FAM167A-BLK gene has been identified as a potential genetic susceptibility locus for dermatomyositis (DM) in patients of European and Japanese populations. Our aim here was to investigate the association between FAM167A-BLK polymorphisms and IIMs risk in Chinese Han. The FAM167A-BLK single nucleotide polymorphisms (SNPs) rs2736340, rs7812879, rs13277113, rs2618479, rs2254546 and rs2248932 were analyzed in polymyositis (PM) patients ($n = 310$), DM patients ($n = 535$) and 968 ethnically matched healthy controls, with the Sequenom MassArray system. Our present study demonstrated that strong allele association was observed in overall PM/DM and PM patients for rs2736340 ($P_c = 6.48 \times 10^{-3}$; $P_c = 0.013$, respectively), rs7812879 ($P_c = 0.017$; $P_c = 0.034$, respectively) and rs13277113 ($P_c = 0.011$; $P_c = 0.047$, respectively). These three SNPs were significantly associated with interstitial lung disease

(ILD) in overall PM/DM patients (all, $P_c < 0.05$). The frequency of the five haplotypes of the five SNPs (rs2736340, rs7812879, rs13277113, rs2618479 and rs2254546) was also significantly different between overall PM/DM, PM or DM patients and healthy controls. This was the first study to demonstrate that the FAM167A-BLK polymorphisms were associated with Chinese PM/DM patients or these patients with ILD, indicating that PM/DM might share common gene with other autoimmune diseases.

Keywords Dermatomyositis · Polymyositis · FAM167A-BLK · Polymorphism · Association

Introduction

The idiopathic inflammatory myopathies (IIMs), collectively named myositis, are characterized by symmetrical, proximal muscle weakness and inflammation in skeletal muscle tissue. Based on their clinical and histopathological features, they may be subdivided into polymyositis (PM), dermatomyositis (DM), inclusion body myositis (IBM) and myositis overlapping with other connective tissue diseases (myositis–CTD overlap syndrome). Besides, PM and DM are the most common subtypes. IIMs are a rare disease in the Caucasian population, with an overall annual prevalence of approximately 10–15 cases per 100,000 individuals [1]. However, its incidence in the Chinese population remains unclear due to the lack of large-scale epidemiological studies. Although IIMs are generally considered as complex multifactorial rheumatic disease, the etiology of IIMs is mostly unknown.

It is supposed that the development of IIMs may be the result of both genetic and environmental factors or their

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interactions. Since IIMs are a rare autoimmune disease, the development of their genetic studies had been restrained [1]. To date, the strongest genetic associations in the IIMs have consistently been shown to be within the major histocompatibility complex (MHC) gene region [2], whereas only a touch of non-MHC loci was identified by genome-wide association study (GWAS) and candidate gene association studies. Non-MHC genes such as tumor necrosis factor alpha (TNF- α) [3–6], interleukin (IL)-1 α , IL-1 β [5], interferon (IFN)- γ [7], interferon-induced helicase (IFIH1) [8], mannose-binding lectin 2 (MBL2) [9], protein tyrosine phosphatase N22 (PTPN22) [10], and signal transducer and activator of transcription 4 (STAT4) [11] confer susceptibility to PM/DM, which indicated that PM/DM shared genetic susceptibility with other autoimmune diseases.

The GWAS [12] had been undertaken on European DM patients. This was the first GWAS with regard to DM, and it revealed that DM share enrichment of genetic loci with other autoimmune diseases. This study confirmed involvement of the MHC region on chromosome 6, although no loci outside the MHC reached genome-wide significance [$P \leq 5 \times 10^{-8}$]. These results identified three genes possibly associated with DM: phospholipase C-like 1 (PLCL1), B lymphoid tyrosine kinase (BLK) and chemokine (C-C motif) ligand 21 (CCL21), none of which had been reported previously in DM. The family with sequence similarity 167 member A-B-lymphoid tyrosine kinase (FAM167A-BLK) region has been well confirmed as susceptibility factors for several autoimmune diseases. Recently, Jani et al. [13] indicated that two SNPs of FAM167A-BLK (rs13277113 and rs2618476) were associated with DM but not PM. Besides, Sugiura et al. [14] showed that rs13277113 in FAM167A-BLK gene was associated with Japanese IIMs patients. In addition, six variants rs2736340, rs7812879, rs13277113, rs2618479, rs2254546 and rs2248932 in the intergenic region between the FAM167A and BLK genes are the common risk factors for systemic lupus erythematosus (SLE) [15–17], rheumatoid arthritis (RA) [18, 19], systemic sclerosis (SSc) [20] and primary sjögren's syndrome (pSS) [21, 22] identified by GWAS and candidate gene association studies in Caucasian and Asian populations. Moreover, upregulation of B cell has been observed in IIMs patients [23], and increased evidence has suggested that auto-reactive B cells play an important role in the pathogenesis of IIMs [24]. Based on FAM167A-BLK gene is involved in the activation and function of B cell, we designed a case–control study to explore the hypothesis that variants of FAM167A-BLK gene might predispose to PM/DM in a Chinese Han population. Our aim was to disclose population differences in genetic variations related in the pathogenesis of PM/DM to improve clinical interference of this disease.

Methods

Subjects

Our research was designed as a large cross-sectional study, and a total of 1819 subjects consisting of 851 PM/DM patients and 968 healthy controls ethnically matched to the cases were recruited in current study. These patients were enrolled from two different sources. Between February 2013 and July 2014, 473 patients including 155 PM patients and 318 DM patients were enrolled from the Peking Union Medical College Hospital. Since this study was supported by the Research Special Fund for Public Welfare Industry of Health, 378 patients containing 155 PM patients and 223 DM patients were recruited through the cooperation of three centers in China.

All patients were ≥ 18 years at the onset of disease and had probable/definite myositis valuated by at least two rheumatologists in light of the criteria of Bohan and Peter [25, 26]. Patients with myositis–CTD overlap syndrome were excluded if they met any of the following published criteria: American College of Rheumatology (ACR) criteria for SLE [27], RA [28], and SSc [29], the American and European consensus criteria for pSS [30] or the criteria for mixed CTD by Sharp et al. [31]. At the same time, we excluded amyopathic dermatomyositis (ADM), who could not meet the traditional criteria of Sontheimer [32]. IBM patients and patients with muscle diseases caused by other factors were systematically excluded. A total of 968 ethnically matched healthy controls were collected from the Peking Union Medical College Hospital during their physical examinations. Our study was approved by the Ethics Committee of the Peking Union Medical College Hospital, and all participants signed a written informed consent.

Selection of SNPs

Given the significant functions of FAM167A-BLK in multiple autoimmune diseases, six SNPs (rs2736340, rs7812879, rs13277113, rs2618479, rs2254546 and rs2248932) of FAM167A-BLK gene, which had previously

Table 1 Clinical data for PM/DM patients and controls

Characteristic	Patients	Controls
Number of subjects (DM/PM)	851 (535/310)	968
Female ratio (%)	74.5	83.7
Average age	45.6 \pm 14.9	43.4 \pm 12.8
DM with ILD, No./total (%)	308/541 (56.9)	–
PM with ILD, No./total (%)	171/310 (55.2)	–

PM polymyositis, DM dermatomyositis, ILD interstitial lung disease

Table 2 Allele and genotype distribution of the BLK gene markers in PM/DM patients and controls

SNPs	Groups	Allele (%)		OR (95 % CI)	P	P _c	Genotype (%)			χ ²	P	P _c
		C	T				CC	CT	TT			
rs2736340	PM	123 (20.2)	487 (79.8)	0.71 (0.57–0.88)	2.24 × 10 ⁻³	0.013	11 (3.6)	101 (33.1)	193 (63.3)	9.41	9.03 × 10 ⁻³	0.054
	DM	239 (22.5)	825 (77.5)	0.81 (0.68–0.97)	0.020	0.120	30 (5.6)	179 (33.7)	323 (60.7)	5.40	0.067	0.402
	PM + DM	362 (21.6)	1312 (78.4)	0.77 (0.66–0.90)	1.08 × 10 ⁻³	6.48 × 10⁻³	31 (3.7)	280 (33.9)	516 (62.4)	10.5	5.28 × 10 ⁻³	0.052
	Controls	508 (26.3)	1424 (73.7)				70 (7.2)	368 (38.1)	528 (54.7)			
rs7812879	PM	105 (17.3)	503 (82.7)	0.72 (0.57–0.91)	6.74 × 10 ⁻³	0.034	TT	TC	CC	7.54	0.023	0.138
	DM	202 (19.0)	860 (81.0)	0.81 (0.67–0.98)	0.029	0.174	8 (2.6)	89 (29.3)	207 (68.1)	4.99	0.082	0.492
	PM + DM	307 (18.4)	1363 (81.6)	0.78 (0.66–0.92)	2.79 × 10 ⁻³	0.017	25 (4.7)	152 (28.6)	354 (66.7)	8.61	0.014	0.084
	Controls	434 (22.4)	1502 (77.6)				33 (4.0)	241 (28.8)	561 (67.2)			
rs13277113	PM	137 (22.5)	473 (77.5)	0.75 (0.60–0.93)	7.77 × 10 ⁻³	0.047	GG	GA	AA	7.23	0.027	0.162
	DM	252 (23.9)	804 (76.1)	0.81 (0.68–0.96)	0.016	0.096	14 (4.6)	109 (35.7)	182 (59.7)	6.06	0.048	0.288
	PM + DM	389 (23.3)	1277 (76.7)	0.79 (0.68–0.91)	1.77 × 10 ⁻³	0.011	35 (6.6)	182 (34.5)	311 (58.9)	9.58	8.30 × 10 ⁻³	0.050
	Controls	540 (27.9)	1394 (72.1)				49 (5.9)	291 (34.9)	493 (59.2)			
rs2618479	PM	124 (20.4)	484 (79.6)	0.77 (0.62–0.97)	0.023	0.138	TT	TC	CC	5.76	0.056	0.336
	DM	228 (21.5)	834 (78.5)	0.82 (0.69–0.99)	0.035	0.210	10 (3.3)	104 (34.2)	190 (62.5)	5.94	0.051	0.306
	PM + DM	352 (21.1)	1318 (78.9)	0.81 (0.69–0.94)	6.69 × 10 ⁻³	0.040	31 (5.8)	166 (31.3)	334 (62.9)	7.50	0.023	0.138
	Controls	482 (24.9)	1454 (75.1)				41 (4.9)	270 (32.3)	524 (62.8)			
rs2254546	PM	111 (18.2)	499 (81.8)	0.77 (0.61–0.97)	0.027	0.162	AA	AG	GG	4.75	0.093	0.558
	DM	215 (20.2)	851 (79.8)	0.87 (0.73–1.05)	0.152	0.912	61 (6.3)	360 (37.2)	547 (56.5)	3.39	0.184	1.104
	PM + DM	326 (19.5)	1350 (80.5)	0.84 (0.71–0.98)	0.029	0.174	AA	AG	GG	5.15	0.076	0.456
	Controls	434 (22.4)	1502 (77.6)				56 (5.8)	322 (33.3)	590 (60.9)			
rs2248932	PM	130 (21.3)	480 (78.7)	0.86 (0.69–1.07)	0.165	0.990	CC	CT	TT	4.88	0.087	0.522
	DM	239 (22.5)	823 (77.5)	0.92 (0.77–1.10)	0.342	2.052	8 (2.6)	114 (37.4)	183 (60.0)	1.55	0.462	2.772
	PM + DM	369 (22.1)	1303 (77.9)	0.89 (0.77–1.05)	0.160	0.960	31 (5.8)	177 (33.3)	323 (60.9)	2.05	0.360	2.160
	Controls	465 (24.0)	1469 (76.0)				39 (4.7)	291 (34.8)	506 (60.5)			

Bold values are statistically significant ($P < 0.05$) (corrected for multiple comparisons by the Bonferroni adjustment test)
 PM polymyositis, DM dermatomyositis, OR odds ratio, CI confidence interval, χ² Chi-square test, P_c P value corrected by Bonferroni method

demonstrated in positive associations with other immune-mediated diseases based on GWAS or candidate gene association studies, were examined in our present study.

Genotyping

Peripheral blood specimen (2 mL) was collected from patients and controls, and DNA of each participant was extracted from peripheral white blood cell by using kits from Tiangen (Beijing, China) and complied with the manufacturer's instructions. The genotyping of all six SNPs from FAM167A-BLK gene was performed using a Sequenom MassArray system (Sequenom iPLEX assay, San Diego, CA, USA) in accordance with the manufacturer's protocol. The primers for polymerase chain reaction (PCR) and for locus-specific single-base extension were designed by the MassArray Assay Design 4.0 (Sequenom). All DNA samples of patients and controls were first transferred to a 384-element plate. After multiplex PCR amplifications, the products were used for locus-specific single-base extension reactions. The final products were subsequently desalted and transferred to 384-element SpectroCHIP array (Sequenom). Allele detection was carried out by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). The resultant mass spectrograms and genotype data were analyzed using MassArray Typer 4.0 software.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) of each SNP was examined by using the Chi-square (χ^2) test in healthy controls, and any SNPs that deviated from the HWE ($P < 0.05$ in the control groups) would be excluded from subsequent analysis. Differences in genotype and allele frequencies between cases and controls were analyzed by the χ^2 test. The odds ratio (OR) and 95 % confidence interval (95 % CI) of associations were calculated, and P values (corrected for multiple comparisons by the Bonferroni adjustment test) < 0.05 were deemed to be statistically significant. For genetic model testing (additive, dominant and recessive model), genotype frequencies were further analyzed using logistic regression models. For the association analysis between FAM167A-BLK polymorphisms and the three clinical subsets (overall PM/DM patients, PM patients and DM patients versus control groups), statistical analysis was calculated by PLINK v1.07 software (Shaun Purcell, Boston, USA) [33]. Stratification analysis with regard to the association study for these six SNPs and the presence of interstitial lung disease (ILD) were performed by the results of the following three comparisons: patients (overall PM/DM patients, PM patients and DM patients) with ILD versus all controls, patients without ILD versus all controls and patients with ILD versus without ILD. The genetic power for this large cross-

Table 3 Analysis of the six SNPs based on three genetic models

SNPs	Groups	Additive model		Dominant model		Recessive model	
		P_c	OR (95 % CI)	P_c	OR (95 % CI)	P_c	OR (95 % CI)
rs2736340	PM	0.015	0.71 (0.57–0.89)	0.049	0.70 (0.54–0.91)	0.157	0.47 (0.25–0.91)
	DM	0.133	0.82 (0.69–0.97)	0.142	0.78 (0.63–0.97)	1.405	0.76 (0.49–1.19)
	PM + DM	7.43×10^{-3}	0.78 (0.67–0.91)	0.016	0.75 (0.62–0.91)	0.239	0.66 (0.44–0.98)
rs7812879	PM	0.047	0.73 (0.58–0.92)	0.138	0.73 (0.55–0.96)	0.222	0.45 (0.21–0.95)
	DM	0.204	0.82 (0.68–0.99)	0.155	0.78 (0.62–0.97)	2.539	0.82 (0.50–1.33)
	PM + DM	0.021	0.79 (0.67–0.92)	0.032	0.76 (0.63–0.92)	0.545	0.68 (0.44–1.06)
rs13277113	PM	0.05	0.75 (0.61–0.93)	0.150	0.74 (0.57–0.96)	0.234	0.54 (0.30–0.97)
	DM	0.109	0.81 (0.69–0.97)	0.089	0.77 (0.62–0.95)	1.705	0.80 (0.53–1.21)
	PM + DM	0.012	0.79 (0.68–0.92)	0.021	0.76 (0.63–0.91)	0.365	0.70 (0.49–1.02)
rs2618479	PM	0.138	0.77 (0.62–0.96)	0.391	0.78 (0.60–1.02)	0.300	0.51 (0.26–1.00)
	DM	0.226	0.83 (0.69–0.99)	0.098	0.77 (0.62–0.95)	4.324	0.92 (0.59–1.44)
	PM + DM	0.044	0.81 (0.69–0.94)	0.043	0.77 (0.64–0.93)	1.220	0.77 (0.51–1.15)
rs2254546	PM	0.182	0.78 (0.62–0.98)	0.300	0.76 (0.58–1.00)	0.846	0.61 (0.32–1.18)
	DM	0.995	0.88 (0.74–1.05)	0.498	0.82 (0.66–1.03)	5.882	1.01 (0.64–1.58)
	PM + DM	0.206	0.84 (0.72–0.99)	0.140	0.80 (0.66–0.97)	2.819	0.86 (0.57–1.30)
rs2248932	PM	0.959	0.85 (0.68–1.07)	2.871	0.91 (0.70–1.18)	0.189	0.44 (0.21–0.93)
	DM	2.076	0.92 (0.77–1.10)	1.440	0.88 (0.71–1.09)	5.822	1.01 (0.64–1.59)
	PM + DM	0.961	0.89 (0.76–1.05)	1.346	0.89 (0.74–1.07)	1.721	0.80 (0.52–1.21)

Bold values are statistically significant ($P < 0.05$) (corrected for multiple comparisons by the Bonferroni adjustment test)

PM polymyositis, DM dermatomyositis, OR odds ratio, CI confidence interval, P_c P value corrected by Bonferroni method

Table 4 Association between the six SNPs and PM/DM with ILD

Disease	Groups	rs2736340			rs7812879			rs13277113			rs2618479			rs2254546			rs2248932		
		P_c	OR (95 % CI)	P_c	OR (95 % CI)	P_c	OR (95 % CI)	P_c	OR (95 % CI)	P_c	OR (95 % CI)	P_c	OR (95 % CI)	P_c	OR (95 % CI)	P_c	OR (95 % CI)		
PM	P versus N	4.89	0.95 (0.64–1.41)	3.08	0.87 (0.57–1.33)	3.87	0.91 (0.62–1.34)	2.85	0.87 (0.58–1.29)	5.13	0.96 (0.64–1.46)	3.80	1.10 (0.74–1.63)						
	P versus C	0.07	0.69 (0.52–0.92)	0.08	0.68 (0.50–0.92)	0.11	0.72 (0.54–0.95)	0.17	0.72 (0.54–0.97)	0.39	0.76 (0.56–1.02)	2.53	0.89 (0.68–1.18)						
DM	N versus C	0.26	0.73 (0.53–0.99)	0.79	0.78 (0.56–1.08)	0.68	0.79 (0.58–1.06)	1.50	0.84 (0.62–1.14)	0.88	0.79 (0.57–1.09)	1.13	0.81 (0.59–1.11)						
	P versus N	2.40	0.88 (0.66–1.18)	2.31	0.87 (0.64–1.19)	1.41	0.84 (0.63–1.12)	2.65	0.89 (0.66–1.20)	4.81	0.96 (0.71–1.30)	3.30	0.92 (0.68–1.22)						
PM + DM	P versus C	0.11	0.77 (0.62–0.96)	0.15	0.77 (0.61–0.97)	0.05	0.75 (0.60–0.93)	0.19	0.78 (0.63–0.98)	1.14	0.86 (0.69–1.08)	1.59	0.88 (0.71–1.10)						
	N versus C	1.51	0.87 (0.69–1.10)	1.84	0.88 (0.68–1.13)	2.00	0.89 (0.71–1.13)	1.81	0.88 (0.69–1.12)	2.28	0.89 (0.70–1.15)	4.58	0.96 (0.76–1.22)						
PM + DM	P versus N	2.53	0.91 (0.72–1.15)	1.70	0.87 (0.68–1.12)	1.34	0.87 (0.69–1.09)	1.80	0.88 (0.70–1.12)	4.59	0.96 (0.76–1.23)	5.11	0.98 (0.78–1.23)						
	P versus C	0.009	0.74 (0.61–0.89)	0.01	0.73 (0.60–0.90)	0.007	0.74 (0.62–0.89)	0.03	0.76 (0.63–0.92)	0.29	0.82 (0.68–1.00)	1.22	0.88 (0.74–1.07)						
PM + DM	N versus C	0.28	0.82 (0.67–1.00)	0.64	0.84 (0.68–1.04)	0.64	0.85 (0.70–1.04)	0.93	0.86 (0.71–1.06)	0.85	0.85 (0.69–1.05)	2.03	0.91 (0.74–1.11)						

Bold values are statistically significant ($P < 0.05$) (corrected for multiple comparisons by the Bonferroni adjustment test)

DM dermatomyositis, PM polymyositis, ILD interstitial lung disease, OR odds ratio, CI confidence interval, P_c P value corrected by Bonferroni method

Group P: patients with ILD; Group N: patients without ILD; Group C: healthy controls; Group P (PM: $n = 171$; DM: $n = 308$; PM + DM: $n = 479$); Group N (PM: $n = 139$; DM: $n = 233$; PM + DM: $n = 372$); Group C ($n = 968$)

sectional study was accomplished with the statistical program Genetic Power Calculator [34]. Haplotype analysis was performed with Haploview software v4.2 [35].

Results

The characteristics of subjects

In the present study, a total of 851 adult-onset PM/DM patients (74.5 % women; mean age 45.6 ± 14.9) were enrolled, including 310 PM patients (73.4 % women; mean age 45.2 ± 14.7) and 535 DM patients (75.6 % women; mean age 46.0 ± 15.1). The ethnically matched healthy controls included 968 subjects (83.7 % women; mean age 43.4 ± 12.8 years). For these patients, 171 of 310 PM patients (55.2 %) and 308 of 541 DM patients (56.9 %) had complicated with ILD. Finally, 479 PM/DM patients had complicated with ILD and 372 patients not. Characteristics of cases and controls engaged in present study are summarized in Table 1. All SNPs in FAM167A-BLK gene were in Hardy–Weinberg equilibrium in controls ($P_{HWE} > 0.05$). The genotype call rates of each SNP were $>97\%$. The power analysis showed that our sample size had more than 80 % power ($\alpha = 0.05$) for detecting association with an OR of 1.10–1.60 for both heterozygotes and homozygotes.

Association of the six SNPs with PM/DM in the Han population

Table 2 summarizes the genotype and allele distribution for the six SNPs in FAM167A-BLK gene. There was statistically significant difference in the allelic and genotypic frequencies in the rs2736340 between overall PM/DM patients and healthy controls ($P_c = 6.48 \times 10^{-3}$ and $P_c = 0.032$; Table 2). In addition, we discovered that the rs2736340 allele was associated with PM development ($P_c = 0.013$). Also, the allele frequencies of rs7812879 and rs13277113 showed statistically significant associations with PM patients and overall PM/DM patients ($P_c = 0.034$ and $P_c = 0.017$; $P_c = 0.047$ and $P_c = 0.011$, respectively). The percentage of overall PM/DM patients with C allele of rs2618479 was significantly higher than that in the healthy controls ($P_c = 0.040$). However, neither of other two SNPs (rs2254546 and rs2248932) demonstrated significant differences in allele or genotype frequencies between patients and controls (all, $P_c > 0.05$).

Further logistic regression analysis was accomplished based upon three genetic models (additive, dominant and recessive model). The outcomes of logistic regression analysis are summarized in Table 3. In the additive and the dominant model, significant associations were observed in PM patients and overall PM/DM patients for rs2736340 ($P_c = 0.015$ and

Table 5 Haplotype analysis of BLK SNPs between DM patients and controls

Groups	Haplotypes					Total of frequency (%)	Case (%)	Control (%)	χ^2	P_c
	rs7812879	rs2254546	rs2736340	rs13277113	rs2618479					
PM	C	A	T	G	C	73.8	74.0	72.1	6.29	0.0122
	T	G	C	A	T	20.9	17.5	22.1	7.33	0.0068
	C	G	C	G	C	2.7	0.8	2.9	6.67	0.0098
	C	G	T	G	T	1.5	1.9	1.8	0.13	0.7203
	C	G	C	G	T	1.1	1.8	1.1	0.20	0.6574
DM	C	A	T	G	C	73.8	76.2	72.2	4.15	0.0415
	T	G	C	A	T	20.8	18.3	21.9	5.58	0.0181
	C	G	C	G	C	2.5	1.9	2.7	2.73	0.0986
	C	G	T	G	T	1.8	1.8	1.9	0.01	0.922
	C	G	C	G	T	1.2	1.8	1.3	0.51	0.4729
PM + DM	C	A	T	G	C	74.3	76.4	72.1	8.18	0.042
	T	G	C	A	T	20.1	17.9	21.9	9.53	0.002
	C	G	C	G	C	2.3	1.8	2.8	7.15	0.0075
	C	G	T	G	T	1.8	2.0	1.9	0.06	0.8079
	C	G	C	G	T	1.5	1.9	1.3	0.54	0.4625

Bold values are statistically significant ($P < 0.05$) (corrected for multiple comparisons by the Bonferroni adjustment test)

PM polymyositis, DM dermatomyositis, P_c P value corrected by Bonferroni method

$P_c = 7.43 \times 10^{-3}$; $P_c = 0.049$ and $P_c = 0.016$, respectively). Besides, associations were observed under the additive and the dominant model for three SNPs (rs7812879, rs13277113 and rs2618479) in overall PM/DM patients (all, $P_c < 0.05$). In addition, rs7812879 indicated weak association with PM patients in the additive model ($P_c = 0.047$). Nevertheless, none of the three genetic models showed any significant differences between patients and controls for two SNPs (rs2254546 and rs2248932) (all, $P_c > 0.05$).

Association between FAM167A-BLK polymorphisms and the ILD phenotype of PM/DM

To analyze FAM167A-BLK polymorphisms in more detail, we further observed whether the associations existed between FAM167A-BLK polymorphisms and ILD phenotype of PM/DM patients. The associations between the six SNPs and patients with/without ILD are shown in Table 4. Notably, the four SNPs (rs2736340, rs7812879, rs13277113 and rs2618479) were associated with overall PM/DM patients with ILD involvement (all, $P_c < 0.05$). However, our present study indicated that the other two SNPs (rs2254546 and rs2248932) were not statistically significantly associated with PM/DM patients with/without ILD in present study (Table 4).

Haplotype analysis of TNFAIP3 SNPs and patients

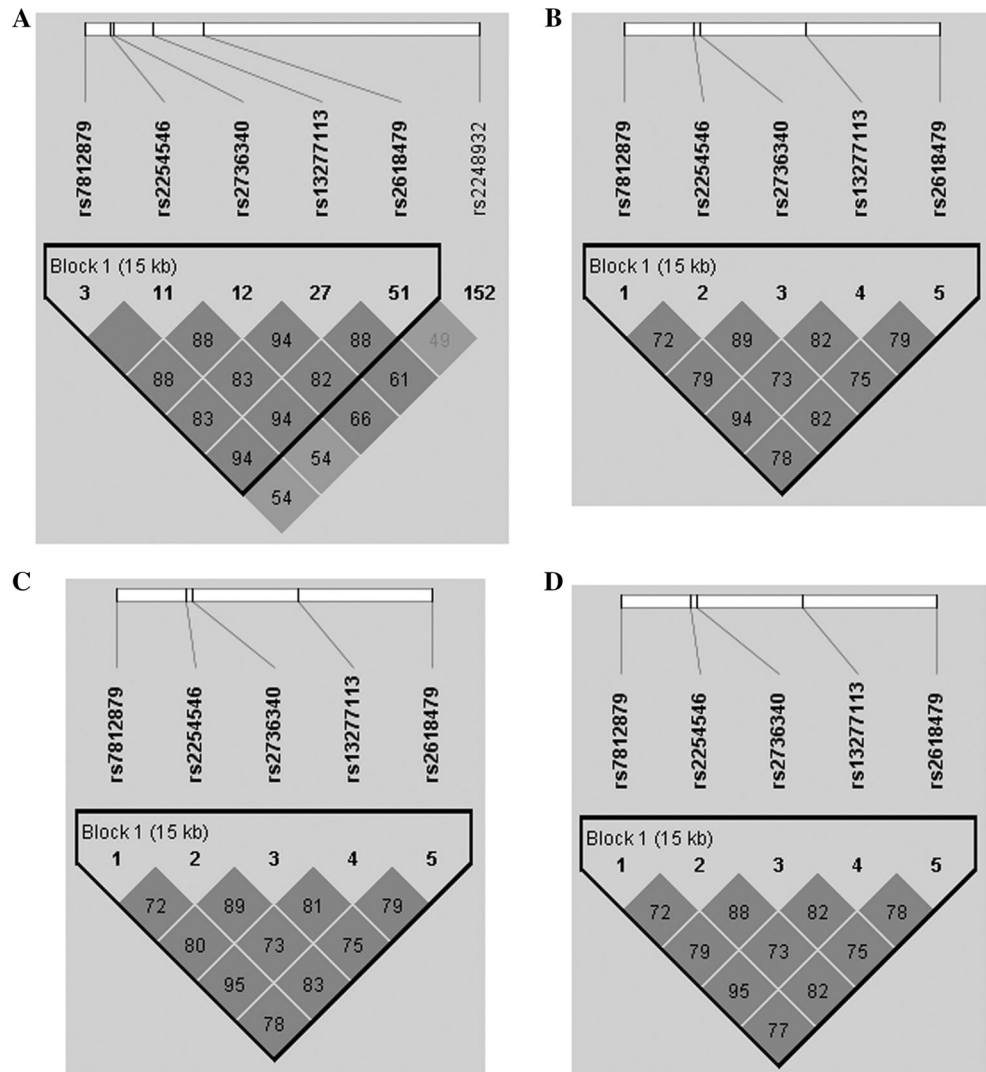
We used Haploview software to further analyze the distributions of the haplotypes in the FAM167A-BLK SNPs

between patients and healthy controls. The results from the LD analysis of the SNPs (rs2736340, rs7812879, rs13277113, rs2618479 and rs2254546) in our study and the data from the HapMap CHB population are shown in Table 5 and Fig. 1. Data from HapMap CHB and the present study illustrated no significant differences. The TGCAT haplotype (rs2736340T–rs7812879G–rs13277113C–rs2618479A–rs2254546T) had a lower frequency between PM, DM or PM/DM patients and healthy controls ($P_c = 0.0068$, $P_c = 0.0181$ and $P_c = 2.0 \times 10^{-3}$, respectively; Table 5).

Discussion

Herein, we first described the association between FAM167A-BLK polymorphisms and PM/DM patients in a Chinese Han population. Our study confirmed that the four SNPs (rs2736340, rs7812879, rs13277113 and rs2618479) in FAM167A-BLK gene region were associated with PM/DM in Chinese Han. It is interesting that even after stratification into traditional IIMs clinical subgroups, the association with PM also presented, but not in patients with DM. The association between FAM167A-BLK polymorphisms (rs2736340, rs13277113 and rs2618479) and IIMs patients was also conducted in Caucasian population [12, 13]. However, Jani et al. [13] indicated that two SNPs of FAM167A-BLK (rs13277113 and rs2618476) were associated with DM but not PM, and the GWAS undertaken on European DM patients also demonstrated that rs2736340

Fig. 1 Linkage disequilibrium (LD) analysis of the SNPs in the FAM167A-BLK gene region. The LD plots were generated by Haploview software v4.2, and data from our study were similar to that from the HapMap CHB population. The number (divided by 100) in the *small square* represents r^2 value and ranges from 0 to 1. The five SNPs (rs2736340, rs7812879, rs13277113, rs2618479 and rs2254546) in FAM167A-BLK reside in an LD block. **a** The data from HapMap CHB. **b** The data analysis between PM patients and healthy controls from our study. **c** The data analysis between DM patients and healthy controls from our study. **d** The data analysis between PM/DM patients and healthy controls from our study



was associated with Caucasian DM patients [12]. Moreover, Sugiura et al. [14] showed that rs13277113 in FAM167A-BLK gene was associated with Japanese IIMs patients, especially in DM patients. Based on the above research and our study, we found the role of FAM167A-BLK gene played in the pathogenesis of DM and PM existed racial and regional differences, which assisted us to improve the clinical interference of this disease.

PM is characterized by symmetric proximal muscle weakness, whereas DM is characterized by an erythematous rash and symmetric proximal muscle weakness. PM and DM are the major clinical subtypes of IIMs, and studies had manifested that the immunopathology of PM and DM is different. DM patients' muscle biopsy demonstrates a mononuclear, inflammatory cell exudate arranged in predominantly perivascular or in the interfascicular septae rather than within the fascicles [36, 37]. On the contrary, PM has a distinct immunohistopathologic phenotype, in which muscle biopsy reveals that the primary

inflammatory cell is lymphocytes (CD8-positive cells) invaded histologically healthy muscle fibers expressing MHC class I antigens [36–38]. Our present study suggested that the role of FAM167A-BLK gene played in the pathogenesis of DM and PM is distinct, presumably because the above immunopathogenesis of PM and DM is inconsistent. At the same time, our study showed that FAM167A-BLK gene polymorphisms were associated with combined cases. In fact, this may be because FAM167A-BLK gene polymorphisms might have high sensitivity for Chinese PM patients, and the relationship between FAM167A-BLK gene polymorphisms and PM patients was found on condition that the sample size of PM patients in our present study was small. Whereas FAM167A-BLK gene polymorphisms might have low sensitivity for Chinese DM patients, the relationship between FAM167A-BLK gene polymorphisms and DM patients was not found on condition that the sample size of PM patients in our present study was relatively large. Interestingly, when we combined the

patients, the association with PM/DM was strong, which owing to the larger sample size. Future studies about PM/DM patients utilizing larger numbers of patients should be executed to confirm these consequences.

The FAM167A is also known as C8orf13, yet the function of FAM167A remains a mystery. However, the BLK is known to encode a non-receptor tyrosine kinase of the src family that is mainly expressed by B cells [39]. The BLK has a role in B cell receptor (BCR) signaling and B cell development by activating the nuclear factor (NF)-kappa B [40]. BCR signaling requires a tight regulation of several protein tyrosine kinases and phosphatases, and associated co-receptors. Binding of antigen to the BCR triggers signaling, which ultimately leads to B cell activation [40]. In the current study, we found that the rs2736340, rs7812879, rs13277113 and rs2618479 variants were associated with the development of PM/DM in Chinese Han population and extended previous finding in other rheumatic diseases, such as SLE [15–17], RA [18, 19], SSc [20] and pSS [21, 22]. Therefore, our study confirmed that PM/DM shares a gene commonly associated with the risk of other autoimmune diseases. Thus, FAM167A-BLK gene may in fact play a foremost role in the pathogenesis of multiple autoimmune diseases as well as to PM/DM.

ILD is the most common complication in patients with IIMs, which has a prevalence of 78 % in IIMs patients. In addition, ILD is the major prognostic factor that contributes to mortality among PM and DM patients. Hence, the investigation with regarding to the relationship between FAM167A-BLK gene polymorphisms and ILD phenotype of PM/DM patients is of great significance. It is also worthwhile to note that the four SNPs (rs2736340, rs7812879, rs13277113 and rs2618479) were associated with PM/DM patients with ILD involvement. It is possible that the rs2736340, rs7812879, rs13277113 and rs2618479 variants may increase the susceptibility to PM/DM by affecting lung function. Therefore, whether PM/DM patients will develop to severe ILD, we might disclose the function of BLK gene, and then, we could predict the prognosis of PM/DM patients. The five SNPs (rs2736340, rs7812879, rs13277113, rs2618479 and rs2254546) resided in the same LD block, and they are the most common susceptibility loci in the FAM167A-BLK gene polymorphisms for other autoimmune diseases. Notably, the TGCAT haplotype was associated with PM, DM and combined PM/DM patients after Bonferroni correction, which confirmed that FAM167A-BLK gene play important role in the pathogenesis of PM/DM.

Because IIMs are rare disease, genetic association studies were always restricted, and early candidate gene studies only included small sample sizes and often divided clinical IIMs subgroups into PM, DM and myositis–CTD overlap syndrome in order to expand statistical power. Notwithstanding, our results were partly inconsistent with

the outcomes of Caucasian and Japanese populations. Our study was the first comprehensive investigation involving patients of PM/DM from Chinese Han, and the clinical features of these patients were consistent with the international guidelines [25, 26]. Importantly, our sample size already has the power (more than 80 %) to examine moderate or even marginal associations. Yet there were some limitations to our present study that should be considered when interpreting our results. For example, we did not examine the function of these genetic variants in the development of PM/DM in these patients.

In conclusion, the present research was the first and comprehensive genetic association analysis of common variants in the FAM167A-BLK gene in Chinese Han population. And our results indicated that FAM167A-BLK gene polymorphisms (rs2736340, rs7812879, rs13277113, rs2618479 and rs2254546) were associated with the risk of DM in Han Chinese population.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The study was approved by the Ethics Committee of the Peking Union Medical College Hospital.

Informed consent Informed consent was obtained from all individual participants included in the study.

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