A single-nucleotide polymorphism of CCL21 rs951005 T>C is associated with susceptibility of polymyositis and such patients with interstitial lung disease in a Chinese Han population

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Abstract

Objective

Our objective was to better understand the roles of single nucleotide polymorphisms (SNPs) in the CCL21, ERBB3, and TERT genes region in the development of idiopathic inflammatory myopathies (IIMs), we explored the associations between SNPs in the mentioned three genes and IIMs susceptibility in a Chinese Han population.

Methods

Chinese polymyositis (PM) patients (n =291), dermatomyositis (DM) patients (n=526) and ethnically-matched healthy controls (n =968) were genotyped for the CCL21 region SNPs (rs951005 and rs2492358), ERBB3 (rs2292239 and rs11171739), and TERT (rs2853676 and rs10069690), by using the Sequenom MassArray system.

Results

Our study indicated strong allele and genotype associations between rs951005 (OR: 1.65, 95%CI: 1.18–2.30, P_C=0.015; P_C=0.041, respectively) in CCL21 gene and PM patients. Additionally, rs951005 was associated with interstitial lung disease (ILD) in PM patients (P_C =0.01), and was associated with PM patients in additive model. However, the Chinese Han PM/DM patients and controls had statistically similar frequencies of alleles, genotypes and different genetic models (additive, dominant, and recessive) of ERBB3 and TERT polymorphisms.

Conclusion

This was the first study to demonstrate that the CCL21 gene SNP (rs951005) might confer genetic predisposition to PM patients or such patients with ILD in a Chinese Han population.

Key words

polymyositis, dermatomyositis, single-nucleotide polymorphisms, CCL21, Han Chinese

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Introduction

The idiopathic inflammatory myopathies (IIMs), commonly known as myositis, are a heterogeneous group of autoimmune disorders including polymyositis (PM), dermatomyositis (DM), inclusion body myositis (IBM) and myositis overlapping with other connective tissue diseases (myositis-CTD overlap syndrome). The most common subtypes are PM and DM, which clinical features are muscle weakness and muscle biopsies typically with inflammatory cell infiltrates. Other than muscle involvement, the lung is the most prevalent and devastating extramuscular manifestation of IIMs (1, 2). The symptom of interstitial lung disease (ILD) has a reported prevalence of 78% in IIMs (3), and is found 65% of newly diagnosed IIMs patients (4). For many years, several MSAs have been identified, and their presence is associated with diverse clinical symptoms and related to the development of ILD, such as anti-synthetase antibodies and anti-MDA5 antibodies (5, 6). Myositis patients complicated with ILD and accompanying malignancies are the major prognostic factors that contribute to mortality among PM and DM patients (7-9). Despite the cause of IIMs still remains unclear, it is supposed to be a combination of both genetic and environmental factors.

The IIMs, regarded as rare autoimmune diseases, have low prevalence (10–15 cases per 100,000), which has restrained the development of genetic studies (10). Recent genome-wide association studies (GWAS) had identified numerous novel susceptibility genes associated with several rheumatic diseases (11, 12). Last year Miller *et al*. (13) conducted a GWAS of adult and juvenile DM patients of European ancestry, which was the first systematic identification of genetic predispositions of DM and promoted the development of DM. This investigation indicated chemokine (C-C motif) ligand 21(*CCL21*) polymorphisms (rs951005 and rs2492358) were the new susceptibility gene loci for DM patients of European ancestry. And the SNPs were previously reported to be associated with rheumatoid arthritis (RA) (14). The *CCL21* is also referred to 6 Ckine or exodus, which involves in recruiting chemokine receptor 7 (CCR7) (+) naive T cells, natural killer, memory T cells, and dendritic cells (DCs) (15-17). The *CCL21* gene, clustering on the p-arm of chromosome 9, is one of the CC cytokine genes. The *CCL21* elicits its effects by binding to a cell surface chemokine receptor known as CCR7, a member of the seven transmembrane-spanning G protein-coupled receptor families, which is expressed on T- and B- lymphocytes and a known receptor for another member of the cytokine family (18-21).

The *ERBB3* (v-erb-b2 avian erythroblastic leukaemia viral oncogene homolog 3) is a member of the epidermal growth factor receptor (EGFR) family of receptor tyrosine kinases (22), which encodes receptor tyrosine kinases erbB-3 (a membrane bound protein), also known as HER3 (human epidermal growth factor receptor 3). The *ERBB3* gene locates on the long arm of chromosome 12 (12q13), and is encoded by 23,651 base pairs, which translates into 1342 amino acids (23). Previously, multiple genetic studies implied that the significant associations existed between *ERBB3* polymorphisms (rs2292239 and rs11171739) and various auto-inflammation diseases such as type 1 diabetes (T1D) (24, 25). At the same time, several articles had proved that PM/DM shared common genetic features with other autoimmune diseases (13, 26-28). The telomerase reverse transcriptase (*TERT*) is a catalytic subunit of the enzyme-telomerase, which, together with the telomerase RNA component (TERC), comprises the most important unit of the telomerase complex (29, 30). To be specific, the *TERT* is responsible for catalysing the addition of nucleotides in a TTAGGG sequence to the ends of a chromosome's telomeres (31). This addition of repetitive DNA sequences prevents degradation of the chromosomal ends from following multiple rounds of replication (32). The *TERT* polymorphisms (rs2853676 and rs10069690) are associated with idiopathic interstitial pneumonia (IIP) (33, 34) and diverse cancer (35, 36) based on GWAS or candidate gene studies. IIP and ILD

in some extent have similar clinical features (37). Therefore, we presumed that IIMs patients with ILD shared a gene commonly associated with the risk of the IIP patients.

However, whether these SNPs in the *CCL21*, *ERBB3*, and *TERT* genes could contribute to the development of PM/DM in Asians has not been explored. Given that these three genes were associated with other autoimmune related diseases, we analysed the potential associations of six SNPs in the *CCL21*, *ERBB3*, and *TERT* genes region with the susceptibility to PM/DM in a Chinese Han population.

Subjects and methods

Study participants

A total of 1, 785 subjects consisting of 817 PM/DM patients and 968 healthy controls ethnically matched to the cases were enrolled in this study. These patients were recruited from two different sources. 439 patients including 136 PM patients and 303 DM patients were collected from the Peking Union Medical College Hospital between February 2013 and May 2014. Since our 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. At the onset all patients were older than 18 years and had probable/definite myositis evaluated by at least two rheumatologists based on the criteria of Bohan and Peter (38, 39). Myositis/CTD-overlap patients were excluded if they satisfied any of the following published criteria (American College of Rheumatology (ACR) criteria for systemic lupus erythematosus (SLE) (40), RA (41), systemic sclerosis (SSc) (42), and American and European consensus criteria for Sjögren's syndrome (SS) (43)) or the criteria for mixed-CTD by Sharp *et al*. (44). We also excluded amyopathic dermatomyositis (ADM), who could not meet the traditional criteria of Sontheimer (45). In addition, IBM and patients muscle diseases caused by other factors were systematically excluded. During physical examinations, we recruited 968 ethnically matched healthy controls from the Peking Union Medical College Hospital according to the following rules:

1) no significant history of rheumatologic disease;
2) no family history of rheumatologic diseases;
3) normal biochemical and immunological profile; and
4) negative serology for anti-Jo-1 and anti-Mi-2 antibodies.

This large case-control study was approved by the Ethics Committee of the Peking Union Medical College Hospital, and all subjects informed in writing consent to be included in present study.

Table I. Clinical data for PM/DM patients and controls.

Characteristic	Patients	Controls
Number of subjects (DM/PM)	817 (526/291)	968
Female ratio (%)	74.7	83.7
Average age	45.9±15.1	43.0±12.6
DM with ILD, n./total (%)	301/526 (57.2)	-
PM with ILD, n./total (%)	160/291 (55.0)	-

PM: polymyositis; DM: dermatomyositis; ILD: interstitial lung disease.

SNP selection and genotyping

Base on the knowledge of the GWAS of adult and juvenile DM patients of European ancestry, the overlapping genes of autoimmune diseases, or the susceptibility genes of PM/DM sub-phenotypes, six SNPs (rs951005, rs2492358, rs2292239, rs11171739, rs2853676 and rs10069690) of *CCL21*, *ERBB3*, and *TERT* genes were selected for further analysis (Table II).

Genomic DNA of all patients and controls were isolated from peripheral white blood cells by using kits from Tiangen (Beijing, China) in accordance with the manufacturer's procedures. The DNA specimens were routinely stored at -20°C. The genotyping of all six SNPs was performed by Sequenom MassArray system with matrix-assisted laser desorption ionisation–time-of-flight mass spectrometry (MALDI-TOF MS; San Diego, CA, USA) according to the manufacturer's instructions. Primers of the six SNPs for the multiplex polymerase chain reaction (PCR) and for locus-specific single-base extension were designed by the MassArray Assay Design 4.0 software. All DNA samples of patients and controls were first transferred to a 384-element plate. After the PCR was carried out, its products were applied to locus-specific single-base extension reactions. The final products were then desalted and transferred to a 384-element SpectroCHIP array (Sequenom). The resultant mass spectrograms and genotypes were analysed by using MassArray Typer software.

Statistical analysis

Each SNP was evaluated for departure from Hardy-Weinberg equilibrium (HWE) by using the Chi-square (χ^2) test in healthy controls. Any SNPs with deviation from the HWE ($p<0.05$ in the control groups) would be excluded from subsequent analysis. The odds ratio (OR) was calculated with 95% confidence interval (95% CI). And p-values less than 0.05 were deemed to be statistically significant. Differences in genotype and allele frequencies between patients and controls were assessed by the χ^2 test. Based on assumptions of logistic regression models, distributions of genotype frequencies were compared by using additive, dominant, and recessive models. For multiple comparisons, a Bonferroni correction was used. For the association analysis between *CCL21*, *ERBB3*, and *TERT* polymorphisms and the three clinical subsets (all PM/DM patients, PM patients and DM patients vs. control subgroups), statistical analysis was calculated by PLINK v1.07 software (Shaun Purcell, Boston, USA) (46). Stratification analysis about the association study for the six SNPs and the presence of ILD was accomplished by the following three comparisons: patients (all PM/DM patients, PM patients and DM patients) with ILD *vs*. without ILD, patients with ILD *vs*. all controls, and patients without ILD *vs*. all controls. The genetic power for our study was evaluated by using the statis-

Table II. Primary information for these SNPs.

Genotyped SNPs	rs951005	rs2492358	rs2292239	rs11171739	rs2853676	rs10069690
Gene	CCL21	CCL21	ERBB3	ERBB3	TERT	TERT
Polymorphism	T>C	T>C	C>A	T>C	G>A	C>T
Chromosome	9	9	12	12	5	5
Function	intron region	intron region	intron region	RPS26-ERBB3	intron region	intron region
Chr Pos (NCBI)	34743684	34737831	56088396	56076841	1288432	1279675
MAF for Chinese in database*	0.089	0.089	0.256	0.278	0.167	0.211
MAF in our controls (n=968)	0.061	NA	0.235	0.247	0.183	0.206
Pc for HWE test in our controls	1.000	NA	0.655	0.863	1.000	0.201
Genotyping method	Sequenom MassArray iPLEX	Sequenom MassArray iPLEX	Sequenom MassArray iPLEX	Sequenom MassArray iPLEX	Sequenom MassArray iPLEX	Sequenom MassArray iPLEX
Genotyping value (%)	99.51	8.36	98.29	99.63	99.02	97.18

NCBI: National Center for Biotechnology Information; MAF: minor allele frequency; * the data were from the International HapMap Project; Pc: *p*-value corrected by Bonferroni method; HWE: Hardy-Weinberg equilibrium; NA: not available.

Table III. Allele and genotype distribution of the CCL21, ERBB3, TERT gene markers in PM/DM patients and controls.

Gene	SNPs	Groups	Genotype (%)			χ^2	*p*	*Pc*	Allele (%)		OR (95%CI)	*p*	*Pc*
			CC	CT	TT				C	T			
CCL21	rs951005	PM	5 (1.70)	46 (15.9)	239 (82.4)	NA*	8.2×10^{-3}	0.041	56 (9.70)	524 (90.3)	1.65 (1.18-2.30)	3.0×10^{-3}	0.015
		DM	0 (0.00)	50 (9.60)	473 (90.4)	NA*	0.48	2.40	50 (4.80)	996 (95.2)	0.77 (0.55-1.09)	0.14	0.70
		PM+DM	5 (0.60)	96 (11.8)	712 (87.6)	NA*	0.63	3.15	106 (6.50)	1520 (93.5)	1.07 (0.82-1.41)	0.60	3.00
		Controls	3 (0.30)	112 (11.6)	853 (88.1)				118 (6.10)	1818 (93.9)			
			AA	AC	CC				A	C			
ERBB3	rs2292239	PM	17 (6.00)	102 (36.0)	164 (58.0)	0.02	0.89	4.45	136 (24.0)	430 (76.0)	1.03 (0.83-1.28)	0.80	4.00
		DM	23 (4.40)	170 (32.7)	327 (62.9)	1.26	0.26	1.30	216 (20.8)	824 (79.2)	0.85 (0.71-1.02)	0.086	0.43
		PM+DM	40 (5.00)	272 (33.9)	491 (61.1)	0.56	0.45	2.25	352 (21.9)	1254 (78.1)	0.91 (0.78-1.07)	0.26	1.30
		Controls	56 (5.80)	343 (35.5)	568 (58.7)				455 (23.5)	1479 (76.5)			
			CC	CT	TT				C	T			
	rs11171739	PM	19 (6.50)	102 (35.1)	170 (58.4)	0.11	0.74	3.70	140 (24.1)	442 (75.9)	0.96 (0.78-1.20)	0.74	3.70
		DM	25 (4.80)	182 (34.8)	316 (60.4)	0.95	0.33	1.65	232 (22.2)	814 (77.8)	0.87 (0.73-1.04)	0.12	0.60
		PM+DM	44 (5.40)	284 (34.9)	486 (59.7)	0.28	0.60	3.00	372 (22.9)	1256 (77.1)	0.90 (0.77-1.05)	0.19	0.95
		Controls	58 (6.00)	363 (37.5)	547 (56.5)				479 (24.7)	1457 (75.3)			
			AA	AG	GG				A	G			
TERT	rs2853676	PM	4 (1.40)	82 (28.3)	204 (70.3)	NA	0.18	0.90	90 (15.5)	490 (84.5)	0.82 (0.64-1.05)	0.12	0.60
		DM	11 (2.10)	150 (28.9)	358 (69.0)	1.69	0.19	0.95	172 (16.6)	866 (83.4)	0.88 (0.72-1.08)	0.23	1.15
		PM+DM	15 (1.80)	232 (28.7)	562 (69.5)	3.61	0.058	0.29	262 (16.2)	1356 (83.8)	0.86 (0.72-1.03)	0.093	0.465
		Controls	32 (3.30)	291 (30.1)	645 (66.6)				355 (18.3)	1581 (81.7)			
			TT	TC	CC				T	C			
	rs10069690	PM	10 (3.50)	79 (27.6)	197 (68.9)	1.35	0.25	1.25	99 (17.3)	473 (82.7)	0.74 (0.58-0.95)	0.02	0.10
		DM	26 (5.10)	147 (28.9)	335 (66.0)	2.20	0.14	0.70	199 (19.6)	817 (80.4)	0.94 (0.78-1.14)	0.53	2.65
		PM+DM	36 (4.50)	226 (28.5)	532 (67.0)	1.19	0.27	1.35	298 (18.8)	1290 (81.2)	0.89 (0.76-1.06)	0.18	0.90
		Controls	34 (3.50)	330 (34.1)	604 (62.4)				398 (20.6)	1538 (79.4)			

PM: polymyositis; DM: dermatomyositis; OR: odds ratio; CI: confidence interval; χ^2: Chi-square test; Pc: P value corrected by Bonferroni method; NA: not available; * the *p*-value of genotypic analysis was calculated under the logistic regression analysis.

tical programme developed by Purcell *et al*. (47).

Results

Characteristics of study participators

Characteristics of cases and controls engaged in present study were summarised in Table I. Briefly, 291 PM patients (73.9% women; mean age: 45.6±14.9 years) and 526 DM patients (75.5% women; mean age: 46.2±15.3 years) were enrolled. Therefore, a total of 817 adult-onset PM/DM patients (74.7% women; mean age 45.9±15.1 years) were collected. Among these patients, 461 PM/DM patients had ILD (160 of 291 PM patients, 55.0%; 301 of 526 DM patients, 57.2%) and 356 patients did not. 968 subjects (83.7% women; mean age 43.0±12.6 years) were enrolled for healthy controls (Table I). The primary information for the six genotyped SNPs was shown in TableII. In all 1785 samples, the success rate of genotyping was more than 97% except for rs2492358 (8.36%), which was excluded from further analysis due to the low call rate. The remaining five SNPs rs951005 (*CCL21*), rs2292239 (*ERBB3*), rs11171739 (*ERBB3*),

Table IV. Analysis of the five SNPs based on three genetic models.

Gene	SNPs	Groups	Additive model		Dominant model		Recessive model	
			Pc	OR (95%CI)	Pc	OR (95%CI)	Pc	OR (95%CI)
CCL21	rs951005	PM	0.019	1.63 (1.17-2.27)	0.06	1.58 (1.11-2.27)	0.09	5.64 (1.34-23.8)
		DM	0.67	0.77 (0.55-1.08)	0.87	0.78 (0.55-1.11)	4.99	NA**
		PM+DM	3.02	1.07 (0.82-1.41)	3.63	1.05 (0.79-1.40)	1.73	1.99 (0.47-8.35)
ERBB3	rs2292239	PM	4.03	1.03 (0.83-1.28)	4.06	1.03 (0.79-1.35)	4.46	1.04 (0.59-1.82)
		DM	0.44	0.85 (0.71-1.02)	0.60	0.84 (0.67-1.05)	1.32	0.75 (0.46-1.24)
		PM+DM	1.30	0.91 (0.78-1.07)	1.52	0.90 (0.75-1.10)	2.27	0.85 (0.56-1.29)
	rs11171739	PM	3.68	0.96 (0.78-1.20)	2.82	0.92 (0.71-1.21)	3.69	1.10 (0.64-1.87)
		DM	0.58	0.87 (0.72-1.04)	0.72	0.85 (0.69-1.06)	1.66	0.79 (0.49-1.28)
		PM+DM	0.94	0.90 (0.77-1.05)	0.87	0.88 (0.73-1.06)	0.94	0.90 (0.77-1.05)
TERT	rs2853676	PM	0.58	0.81 (0.63-1.05)	1.18	0.84 (0.63-1.12)	0.47	0.41 (0.14-1.17)
		DM	1.13	0.88 (0.72-1.08)	1.79	0.90 (0.71-1.13)	0.98	0.63 (0.32-1.27)
		PM+DM	0.44	0.86 (0.72-1.02)	1.01	0.88 (0.72-1.07)	0.31	0.55 (0.30-1.03)
	rs10069690	PM	0.09	0.73 (0.57-0.95)	0.11	0.72 (0.54-0.95)	1.25	0.60 (0.25-1.44)
		DM	2.66	0.94 (0.78-1.14)	0.89	0.86 (0.68-1.07)	0.70	1.48 (0.88-2.50)
		PM+DM	0.92	0.89 (0.76-1.06)	0.22	0.82 (0.67-1.00)	1.38	1.30 (0.81-2.11)

PM: polymyositis; DM: dermatomyositis; OR odds ratio; CI confidence interval; Pc: *p*-value corrected by Bonferroni method; NA: not available; ** The CC genotype frequencies of rs951005 were too low to carry out recessive genetic model analysis in PM patients.

Table V. Association between the five SNPs and PM/DM with ILD.

Disease	Groups	rs951005 (CCL21)		rs2292239 (ERBB3)		rs11171739 (ERBB3)		rs2853676 (TERT)		rs10069690 (TERT)	
		Pc	OR (95%CI)	Pc	OR (95%CI)	Pc	OR (95%CI)	Pc	OR (95%CI)	Pc	OR (95%CI)
PM	P vs. N	1.90	1.29(0.73-2.26)	0.19	0.66(0.45-0.98)	0.06	0.61(0.42-0.90)	3.00	1.13(0.72-1.78)	0.45	1.47(0.94-2.29)
	P vs. C	0.01	1.83(1.23-2.74)	1.32	0.85(0.63-1.14)	0.33	0.76(0.57-1.02)	1.85	0.86(0.63-1.19)	3.70	0.95(0.71-1.28)
	N vs. C	0.71	1.42(0.89-2.29)	0.53	1.27(0.95-1.70)	0.68	1.24(0.93-1.66)	0.71	0.77(0.53-1.10)	0.09	0.65(0.45-0.93)
DM	P vs. N	1.54	0.74(0.42-1.31)	2.87	1.09(0.80-1.48)	4.71	1.01(0.75-1.36)	3.12	1.09(0.78-1.52)	4.50	1.02(0.75-1.40)
	P vs. C	0.39	0.67(0.43-1.05)	1.39	0.88(0.71-1.10)	1.07	0.87(0.70-1.08)	2.38	0.92(0.72-1.17)	3.31	0.95(0.75-1.20)
	N vs. C	3.32	0.91(0.58-1.41)	0.53	0.81(0.63-1.05)	1.18	0.86(0.67-1.10)	1.15	0.84(0.64-1.12)	2.94	0.93(0.72-1.21)
PM+DM	P vs. N	4.42	0.97(0.65-1.44)	1.93	0.90(0.71-1.14)	0.66	0.84(0.66-1.06)	2.35	1.10(0.84-1.44)	1.32	1.15(0.90-1.49)
	P vs. C	3.60	1.06(0.77-1.47)	0.78	0.87(0.72-1.05)	0.27	0.83(0.69-1.00)	1.55	0.90(0.73-1.11)	3.06	0.95(0.78-1.16)
	N vs. C	3.10	1.09(0.77-1.55)	3.75	0.97(0.79-1.19)	4.80	0.99(0.81-1.22)	0.42	0.81(0.64-1.03)	0.43	0.82(0.66-1.03)

DM: dematomysitis; PM: polymyositis; ILD: interstitial lung disease; Group P: patients with ILD; Group N: patients without ILD; Group C: Healthy controls; Pc: *p*-value corrected by Bonferroni method. Group P (DM: n=301; PM: n=160; DM+PM: n=461); Group N (DM: n=225; PM: n=131; DM+PM: n=356); Group C (n=968).

rs2853676 (*TERT*) and rs10069690 (*TERT*) in the controls were in HWE. The concordance rates of repeated analyses reached 100%. As for the minor allele frequency (MAF), there was no noticeable difference between our controls and database of Chinese subjects for these remaining five SNPs (Table II). The power analysis revealed that our sample size had more than 80% power (α=0.05) for detecting association with an OR of 1.10–1.60 for both heterozygotes and homozygotes.

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

Table III summarises the genotype and allele distribution for these remaining five SNPs (rs951005, rs2292239, rs11171739, rs2853676 and rs10069690). Only rs951005 in *CCL21* gene region illustrated suggestive associations with PM patients when allele and genotype frequencies were analysed. (P_C=0.041 and P_C=0.015, respectively). However, for the *ERBB3* and *TERT* genes region, none of the four SNPs (rs2292239, rs11171739, rs2853676 and rs10069690) demonstrated significant differences in allele or genotype frequencies between patients and controls (all, P_C>0.05; Table III). Statistical analysis by using multiple logistic regression in genetic additive, dominant, and recessive models was then conducted. As shown in Table IV, for rs951005 in *CCL21* gene region, significant association was observed in PM patients in the additive model (P_C=0.019) and weak association was signified in PM patients under the dominant model (P_C=0.060). None of the three genetic models manifested any significant difference between cases and controls for the two SNPs (rs2292239 and rs11171739) of *ERBB3* gene (all, P_C>0.05; Table IV) and the two SNPs (rs2853676 and rs10069690) in *TERT* gene (all, P_C>0.05; Table IV).

Association between CCL21, ERBB3, and TERT polymorphisms and the ILD phenotype of PM/DM

In order to analyse *CCL21, ERBB3,* and *TERT* polymorphisms in more detail, we further examined whether the associations existed between *CCL21, ERBB3,* and *TERT* polymorphisms and ILD phenotype of PM/DM patients. The detailed information of the asso-

ciations was showed in Table V. Notably, in *CCL21* gene region, there was a statistically significant difference of rs951005 between PM patients with ILD and healthy controls (P_C=0.01). However, our present study indicated rs2292239 and rs11171739 in *ERBB3* gene and rs2853676 and rs10069690 in *TERT* gene region were not statistically significant associated with PM/DM patients with/without ILD in present study (Table V).

Discussion

In this hospital-based case-control study of PM/DM, we investigated the association of *CCL21* rs951005 T>C, *CCL21* rs2492358 T>C, *ERBB3* rs2292239 C>A, *ERBB3* rs11171739 T>C, *TERT* rs2853676 G>A and *TERT* rs10069690 C>T polymorphisms with risk of PM/DM in a Chinese population. In present study, our consequences demonstrated that *CCL21* rs951005 T>C polymorphism exhibited significant correlation to PM patients. In addition, significant association was also noticed among PM patients with ILD. To our knowledge, this is the first study demonstrating a significant association between the *CCL21* rs951005 T>C polymorphism with the susceptibility of PM in a Chinese Han population.

The *CCL21* expression has been shown to be localised in high endothelial venules (HEVs) in lymph nodes under physiological conditions (48) as well as in non-lymphoid tissues under inflammatory conditions (49). For example, the *CCL21* is also expressed on plasmacytoid dendritic cells, which are important sources of the IFN signature seen in both adult and juvenile PM/DM (50). Additionally, the *CCL21* is also expressed on mononuclear cells in muscle (non-lymphoid tissues) of juvenile DM (51). Similar to other chemokine, the *CCL21* protein inhibits haemopoiesis, stimulates chemotaxis *in vitro* for thymocytes and activates T cells, but not for B cells, macrophages, or neutrophils (52). The *CCL21* cytokine may also play a role in mediating homing of lymphocytes to secondary lymphoid organs in angiogenesis (53) and in B cell migration and proliferation (54) in RA. The *CCL21* rs951005 T>C polymorphism had been reported to be associated with RA (14) based on the GWAS investigation. Recently the GWAS undertaken on DM demonstrated association of rs951005 with DM in European subjects (13). But our present study indicated that the *CCL21* rs951005 T>C polymorphism was associated with PM patients, not DM patients. The reason for this difference may be somewhat as follows. Firstly, this polymorphic marker showed distinct patterns of genetic contribution to IIMs across different racial groups. Secondly, the immunopathology of PM and that of DM were not the same. Investigations had revealed that PM muscle biopsy is characterised by $CD8^+$ T-cell-mediated cytotoxicity against the major histocompatibility complex (MHC) class I antigens expressed by muscle fibers. This is in contrast to DM, in which $CD4^+$ T-cells and B-cells are known to predominate in the perivascular areas of the muscle tissue, and in which complement-mediated injury directed against the interfascicular septae is thought to be more significant (55). Thirdly, our sample size of PM patients is relatively small. Therefore, future studies about *CCL21* rs951005 T>C polymorphism of PM/DM patients with larger sample sizes should be performed to confirm these outcomes in different ethnic subjects.

The *ERBB3* is expressed in skin, bone, muscle, nervous system, heart, lungs, and intestinal epithelium (56). The *ERBB3* also participates in the activation of the phosphatidylinositol 3 kinase/protein kinase B (PI3K/Akt) signaling pathway. The intracellular domain of *ERBB3* contains 6 recognition sites for the SH2 domain of the p85 subunit of PI3K (57). The *ERBB3* binding to the ligands causes the allosteric activation of p110, which is the lipid kinase subunit of PI3K (58), and a function not found in either EGFR or ERBB2. While no evidence has been found that *ERBB3* overexpression, constitutive activation, or mutation alone is oncogenic, the protein as a heterodimerisation partner, most critically with ERBB2, is implicated in growth, proliferation, chemotherapeutic resistance, and the promotion of invasion and metastasis (59). The *TERT* is a catalytic subunit of the enzyme-telomerase. The enzyme consists of a protein component with reverse transcriptase activity, and is an RNA component that serves as a template for the telomere repeat. In fact, there is a strong correlation between telomerase activity and malignant tumours or cancerous cell lines (60). Not all types of human cancer have increased telomerase activity. 90% of cancers are characterised by increased telomerase activity (60). Lung cancer is the most well characterised type of cancer associated with telomerase (36). Despite that *ERBB3* polymorphisms were associated with other autoimmune diseases (24, 25) and that *TERT* polymorphisms had interactive effect with IIP (33, 34), which in some extent have similar clinical features with the sub-phenotype of PM/DM (37), the data from our study had demonstrated that the polymorphisms in *ERBB3* and *TERT* had no genetic correlation to PM/DM in Chinese populations. And analysis by phenotype stratification in our investigation gave no positive signal to indicate correlation to PM/DM with ILD. This finding may be due to the lack of association between PM/DM and *ERBB3* and *TERT*. In addition, differences in the genetic background of diseases may account for our results. Future studies performed by different ethnic groups might reveal whether SNPs in *ERBB3* and *TERT* genes are associated with susceptibility to PM/DM.

In conclusion, the strong correlation had been first observed between *CCL21* rs951005 T>C polymorphism and PM patients in a Chinese Han population. Although our study had larger sample size than previous candidate genetic association study performed for PM/DM, it was still limited. More researches are required to further explore the associations of *ERBB3* and *TERT* with PM/DM.

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