Genetic Association Study of TNFAIP3, IFIH1, IRF5 Polymorphisms with Polymyositis/Dermatomyositis in Chinese Han Population

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Abstract

**Background:** Single-nucleotide polymorphisms (SNPs) in the TNFAIP3, IFIH1, and IRF5 genes have been associated with several auto-inflammation diseases, while the susceptibility between these genes and idiopathic inflammatory myopathies (IIMs) were not reported. This study aimed to investigate whether TNFAIP3, IFIH1, and IRF5 gene polymorphisms confer susceptibility for the IIMs in Chinese Han population.

**Methods:** A large case–control study of Chinese subjects with polymyositis (PM) (n = 298) and dermatomyositis (DM) (n = 530) was accomplished. 968 healthy and ethnically matched controls were available for comparison. Six SNPs in the TNFAIP3 region (rs2230926 and rs5029939), the IFIH1 gene (rs1990760 and rs3747517) and the IRF5 region (rs4728142 and rs729302) were assessed and genotyped using the Sequenom MassArray iPLEX platform.

**Results:** Our study indicated a strong allele association was observed in PM/DM and PM patients for rs2230926 (OR: 1.61, 95%CI: 1.20–2.16; P = 7.5×10^{-3}; OR: 1.88, 95%CI: 1.30–2.74; P = 4.0×10^{-3}, respectively) and rs5029939 (OR: 1.64, 95%CI: 1.21–2.11; P = 6.0×10^{-3}; OR: 1.88, 95%CI: 1.28–2.76; P = 5.5×10^{-3}, respectively). And rs2230926 and rs5029939 were significantly associated with interstitial lung disease (ILD) in PM/DM and PM patients (P = 0.026 and P = 0.016; P = 0.02 and P = 0.03, respectively). In addition, rs4728142 allele and genotype had significant association with PM/DM patients (P = 0.026 and P = 0.048, respectively). Further analysis with three logistic regression genetic models revealed statistically significant difference in the genotypic distribution in the PM/DM, PM or DM patients when the additive and dominant models were used.

**Conclusions:** This was the first study to reveal TNFAIP3 and IRF5 polymorphisms were associated with PM/DM patients or these patients with ILD, indicating that TNFAIP3 and IRF5 might be the susceptibility gene for PM/DM patients in Chinese Han population.

Citation: Chen S, Wang Q, Wu Z, Li Y, Li P, et al. (2014) Genetic Association Study of TNFAIP3, IFIH1, IRF5 Polymorphisms with Polymyositis/Dermatomyositis in Chinese Han Population. PLoS ONE 9(10): e110044. doi:10.1371/journal.pone.0110044

Editor: Xin-Yuan Guan, The University of Hong Kong, China

Received July 30, 2014; Accepted September 5, 2014; Published October 22, 2014

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported by funding from the Research Special Fund for Public Welfare Industry of Health (201202004), and the National Natural Science Foundation of China Grants (81172857, 81373188), the Chinese National High Technology Research and Development Program, Ministry of Science and Technology Grants (2011AA02A113), and the National Science Technology Pillar Program in the 12nd Five-year Plan (2014BAI07B00). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

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Introduction

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of rare systemic disease characterized by skeletal muscle weakness and presented with extra-muscular manifestations such as skin rashes, interstitial lung disease (ILD) and malignancy [1]. The primary subgroups of IIMs are polymyositis (PM), dermatomyositis (DM) and inclusion-body myositis (IBM). Although IIMs are generally considered as multi-factorial autoimmune disease, the etiology of IIMs remains mostly unclear. Myositis patients may develop additional rheumatic diseases, and the occurrence of autoimmune disorders in near relatives is higher [2–3]. According to the understanding of other autoimmune diseases, it is supposed that IIMs development may be a result of both genetic and environmental factors or their interactions. Therefore, it is possible to make use of the extensive knowledge of other rheumatic diseases that share pathogenic traits with IIMs to obtain insight into the probable genetic intricacy of IIMs.

To date, the abundant evidence demonstrated that substantial genetic risk for IIMs existed within the major histocompatibility
complex (MHC) gene region [4], whereas only a handful of non-MHC loci were identified by genome-wide association study (GWAS) and candidate gene association studies in Japanese and European populations [3–15]. Tumor necrosis factor alpha (TNF-α) [5–8], interleukin (IL)-1α, IL-1β [7], interferon (IFN)-γ [9], interferon-induced helicase (IFIH1) [10], mannose-binding lectin 2 (MBL2) [11], protein tyrosine phosphatase N22 (PTPN22) [12], and signal transducer and activator of transcription 4 (STAT4) [13], and other genes were indicated to associate with the risk of IIMs development. Furthermore, recently GWAS [14] had been undertaken on European DM patients. This study showed phospholipase C-like 1 (PLCL1) gene, B lymphoid tyrosine kinase (BLK) gene, and chemokine (C-C motif) ligand 21 (CCL21) genes were associated with DM risk. This was the first GWAS with regard to DM, and it confirmed the MHC as the major genetic region associated with DM and revealed DM share enrichment of genetic loci with other autoimmune diseases.

The TNF-α-induced protein 3 (TNFAIP3) gene, located on chromosome 6q23, participates in nuclear factor (NF-κB) signaling pathways. It is known that genetic variants of the TNFAIP3 gene loci were associated with susceptibility to multiple autoimmune and inflammatory diseases including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), sjogren’s syndrome (SS), systemic sclerosis (SSc), psoriasis (PsA) and inflammatory bowel diseases (IBDs) [15–17]. Of these gene variants, two SNPs (rs2230926 and rs5029939) were mostly widely investigated, and associated with diverse rheumatic diseases [15–17]. Chinoy et al. [18] manifested NF-κB-related gene (IKB1L62) may confer susceptibility to IIMs. The interferon-induced helicase (IFIH1) gene, also known as melanoma differentiation-associated 5 (MDA5), is located at the chromosome 2q24.3. So far, numerous of case-control studies had been conducted to assess the associations of the IFIH1 rs1990760 and rs3747517 polymorphisms with many kinds of autoimmune diseases [19–22]. Gono et al. [10] reported the IFIH1 rs1990760 “AA” genotype might be a risk factor for the onset of ILD with PM in the Japanese population. The interferon regulatory factor 5 (IRF5) gene is located on human chromosome 7q32 and contains nine exons. IRF5 is fundamentally expressed in almost all lymphoid organs (except thymus), especially in B cells, monocytes plasmacytoid dendritic cells and monocyte-derived dendritic cells [23–24]. In recent decades, a number of studies had indicated IRF5 gene displayed a strong association with varied autoimmune diseases [25–27]. The IFIH1 gene and IRF5 gene both participate in type I interferon (IFN) signaling pathway. Previously, the microarray studies revealed that IFN pathway was involved in the pathogenesis of DM and observed up-regulated in muscle tissue, skin tissue and peripheral blood cells [28–30]. The up-regulation of IFN pathway may be a more sensitive marker of disease activity in DM.

Considering the roles of TNFAIP3, IFIH1, and IRF5 in innate and cell-mediated immunity and the reported associations with several autoimmune diseases, we hypothesized that some of the related polymorphisms of TNFAIP3, IFIH1, and IRF5 gene might be part of the genetic background that results in the development of IIMs in a Chinese Han population.

**Methods**

**Subjects**

This study was designed as a large cross-sectional study, and we recruited 286 PM patients and 355 DM patients from two different sources. Between February 2013 and May 2014, 143 PM patients and 307 DM patients were enrolled from the Peking Union Medical College Hospital. Supported by the Research Special Fund for Public Welfare Industry of Health, 155 PM patients and 223 DM patients were recruited through the cooperation of three centers in China. Finally, 828 PM/DM patients were collected in our study. All patients were 18 years or older at the onset of disease and had probable or definite myositis assessed by at least two rheumatologists according to the criteria of Bohan and Peter [31–32]. Patients with myositis–CTD overlap syndrome were excluded if they met either the following published criteria (American College of Rheumatology (ACR) criteria for SLE [33], ACR criteria for RA [34], ACR criteria for SSc [35] and American and European consensus criteria for SS [36]) or the criteria for mixed CTD by Sharp et al [37]. And we also excluded amyopathic dermatomyositis (ADM), who could not meet the traditional criteria of Sontheimer [38]. As IBM is much less prevalent among non-Caucasian than Caucasian populations, IBM patients were not enrolled. Patients with myasthenia gravis, myasthenia syndrome, muscular dystrophy, inherited, metabolic, or infectious myopathies or muscle diseases caused by other factors were systematically excluded. 968 ethnically matched healthy controls from the Peking Union Medical College Hospital were recruited during their physical examinations according to the following rules: 1) no significant history of rheumatologic disease; 2) no family history of rheumatologic diseases; 3) normal biochemical and immunological profiles; and 4) negative serology for anti-Jo-1 and anti-Mi-2 antibodies. This 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 dominant functions of TNFAIP3, IFIH1, and IRF5 in the autoimmune diseases, 6 SNPs (rs2230926, rs5029939, rs1990760, rs3747517, rs4728142 and rs729302) of these genes, which had previously illustrated in a positive association with other immune-mediated diseases based on GWAS or candidate gene studies, were used for further analysis. The information of each SNP was described in Table S1 in the File S1.

**Genotyping**

DNA of all patients and controls were extracted from peripheral white blood cells by using kits from Biotek (Beijing, China) and following the manufacturer’s instructions. The DNA of each participant was genotyped using Sequenom MassArray system (San Diego, CA, USA) according to the manufacturer’s protocol. Primers for the multiplex polymerase chain reaction (PCR) and for locus-specific single-base extension were designed by the MassArray Assay Design 4.0 software. The PCR was carried out in a 384 plate, and the products were used for locus-specific single-base extension reactions. The final products were then desalted and transferred to a 384-element SpectroCHIP array (Sequenom, CA). Allele detection was performed by matrix-assisted laser desorption ionization–time-of-flight mass spectrometry (MALDI-TOF MS). The resultant mass spectromet data were analyzed using MassArray Typer software.

**Statistical analysis**

For the association analysis between TNFAIP3, IFIH1, and IRF5 polymorphisms and the three clinical subgroups (all PM/DM patients, PM patients and DM patients vs. control), statistical analysis was accomplished by PLINK v1.07 software (Shaun Purcell, Boston, USA) [39]. The Hardy–Weinberg equilibrium (HWE) in healthy controls was evaluated by using the Chi-square (χ²) test for these six SNPs. Any SNPs that deviated from the HWE (P<0.05 in the control groups) would be excluded from subsequent analysis. Genotype and allele distribution between
patients and controls were analyzed by the χ² test, and P values (corrected for multiple comparisons by the Bonferroni adjustment test) less than 0.05 were regarded as statistically significant. The odds ratio (OR) of associations was calculated with 95% confidence interval (95% CI). For genetic model testing (additive model, dominant model, and recessive model), genotype frequencies were further analyzed using logistic regression models. Subphenotype stratification analysis with regard to the association study for these six SNPs and the presence of ILD was carried out by the results of the following three comparisons: all PM/DM patients, PM patients and DM patients) with ILD vs. all controls, patients without ILD vs. all controls, and patients with ILD vs. without ILD. The genetic power for this case-control study was calculated with the statistical program Genetic Power Calculator [40]. Haplotype analysis was performed by Haplovew software v4.2 [41].

Results

Characterization of study subjects

The fundamental characteristics of all the participators were summarized in Table 1. In present study, 298 PM patients (73.6% women) and 530 DM patients (76.6% women) were enrolled. The mean ages for PM patients and DM patients were 45.6±14.9 and 46.8±15.5 years, respectively. In a word, a total of 828 adult-onset PM/DM patients (75.1% women; mean age 46.2±12.6 years) were collected. For these patients, 166 of 298 PM patients (55.7%) and 297 of 530 DM patients (56.0%) had ILD. Finally, 460 PM/DM patients (75.1% women; mean age 46.2±12.6 years) were analyzed. According to the results of the following three comparisons: PM patients or PM/DM patients with ILD involved (ILD vs. without ILD) and 361 patients not. The average age of PM patients and DM patients were 43.1 years and 12.6 years, respectively. In word, a total of 828 adult-onset PM/DM patients (75.1% women; mean age 46.2±12.6 years) were enrolled. The mean ages for PM patients and DM patients were 45.6±14.9 and 46.8±15.5 years, respectively. In a word, a total of 828 adult-onset PM/DM patients (75.1% women; mean age 46.2±12.6 years) were collected. For these patients, 166 of 298 PM patients (55.7%) and 297 of 530 DM patients (56.0%) had ILD. Finally, 460 PM/DM patients (75.1% women; mean age 46.2±12.6 years) were analyzed. According to the results of the following three comparisons: PM patients or PM/DM patients with ILD involved (ILD vs. without ILD) and 361 patients not. The average age of PM patients and DM patients were 43.1 years and 12.6 years, respectively.

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

Table 2 summarized the genotype and allele frequencies for these five SNPs (rs2230926, rs5029939, rs1990760, rs3747517 and rs4728142). For the TNFAIP3 region, the rs2230926 allele and genotype were associated with PM patients or PM/DM patients \((P_c = 4.0×10^{-3}\) and \(P_r = 0.02\); \(P_c = 7.5×10^{-3}\) and \(P_r = 0.04\), respectively). And rs5029939 showed a significant association with PM patients or PM/DM patients \((P_c = 5.5×10^{-3}\) and \(P_r = 6.0×10^{-3}\), respectively) when allele frequencies were analyzed for the IFIH1 gene, region, neither of the two SNPs (rs1990760 and rs3747517) demonstrated significant differences in allele or genotype frequencies between patients and controls (all \(P_r > 0.05\)). For the IRF5 gene, the genotype frequencies of rs4728142 manifested associations with DM patients or PM/DM patients \((P_c = 0.042\) and \(P_r = 0.048\), respectively). Additionally, the percentage of PM/DM patients with A allele of rs4728142 was significantly higher than that in the healthy controls \((P_r = 0.026\).

Further logistic regression analysis was performed based upon three genetic models (additive, dominant, and recessive model). The analysis outcomes of these three models were summarized in Table 3. In the additive and the dominant model, significant associations were observed in PM patients or PM/DM patients for two SNPs (rs2230926 and rs5029939) in TNFAIP3 gene region (all, \(P_r < 0.05\)). None of the three genetic models showed any significant differences between cases and controls for two SNPs (rs1990760 and rs3747517) of IFIH1 gene (all, \(P_r > 0.05\)). For rs4728142 in IRF5 gene region, associations were also observed under the additive and the dominant model in PM/DM patients (all, \(P_r < 0.05\)). In additional, rs4728142 indicated weak association with DM patients in the additive model.

Association between TNFAIP3, IFIH1, and IRF5 polymorphisms and the ILD phenotype of PM/DM

Table 1. Clinical data for PM/DM patients and controls.

| Characteristic | Patients | Controls |
|---------------|----------|----------|
| Number of subjects (DM/PM) | 828(530/298) | 968 |
| Female ratio (%) | 83.7 | 75.1 |
| Average age | 46.2±15.2 | 43.1±12.6 |
| DM with ILD, No/total (%) | 297/530(56.0) | - |
| PM with ILD, No/total (%) | 166/298(55.7) | - |

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

doi:10.1371/journal.pone.0110044.t001

Then, it had also been performed that the association analysis between TNFAIP3, IFIH1, and IRF5 polymorphisms and ILD phenotype of PM/DM patients. The associations between these five SNPs and PM/DM patients with/without ILD were summarized in Table 4. Significantly, rs5029939 in TNFAIP3 gene was associated with PM patients or PM/DM patients with ILD involvement \((P_c = 0.03\) and \(P_r = 0.02\), respectively). Similarly, there was a statistically significant difference in rs2230926 between PM patients or PM/DM patients with ILD and healthy controls \((P_c = 0.016\) and \(P_r = 0.04\), respectively). However, rs1990760, rs3747517 in IFIH1 gene and rs4728142 in IRF5 gene region were not statistically significant associated with PM/DM patients with/without ILD in present study.

Haplotype analysis of TNFAIP3 SNPs and patients

We used Haplovew software to further analyze the distributions of the haplotypes in the TNFAIP3 SNPs between patients and healthy controls. The results from the LD analysis of the SNPs (rs2230926, rs5029939) in our study and the data from the HapMap CHB population were shown in Table 5 and Fig. 1. Data from HapMap CHB and the present study illustrated no significant differences. And strong LD association existed between
Table 2. Allele and genotype distribution of the TNFAIP3, IRF5, IFIH1 gene markers in PM/DM patients and controls.

| Gene   | SNPs                  | Groups | Allele (%) | Genotype (%) | OR (95%CI) | P   | Pc  | Genotype | OR (95%CI) | P   | Pc  |
|--------|-----------------------|--------|------------|--------------|------------|-----|-----|----------|------------|-----|-----|
| TNFAIP3| rs2230926             | DM     | G 63(61)   | T 967(93.9)  |            | 1.45(1.04–2.03) | 0.03 | 0.15 | GG 4(0.8) | 55(10.7)   | 456(88.5) | NA* 0.06 | 0.30 |
|        |                       | PM     | G 45(7.8)  | T 533(92.2)  | 1.88(1.30–2.74) | 8.0×10⁻⁴ | 4.0×10⁻¹ | GT 2(0.7) | 41(14.2)   | 246(85.1) | NA* 4.0×10⁻¹ | 0.02 |
|        |                       | DM+PM  | G 108(6.7) | T 1500(93.3) | 1.61(1.20–2.18) | 1.5×10⁻³ | 7.5×10⁻² | TT 6(0.7) | 96(12.0)   | 702(87.3) | NA* 7.7×10⁻² | 0.04 |
|        |                       | Controls| G 83(4.3)  | T 1851(95.7) |            |            |      |      | 1(0.1)   | 81(8.4)    | 885(91.5) |        |     |
|        |                       |        |            |              |            |            |      |      |          |            |        |     |
|        | rs5029939             | DM     | G 62(6.0)  | T 968(94.0)  | 1.50(1.07–2.11) | 0.02 | 0.10 | GC 3(0.6) | 56(10.9)   | 456(88.5) | NA* 0.22 | 1.10 |
|        |                       | PM     | G 43(7.4)  | T 537(92.6)  | 1.88(1.28–2.76) | 1.1×10⁻³ | 5.5×10⁻² | GC 0(0.0) | 43(14.8)   | 247(85.2) | NA* NA* NA* |     |
|        |                       | DM+PM  | G 105(6.5) | T 1505(93.5) | 1.64(1.21–2.21) | 1.2×10⁻³ | 6.0×10⁻² | GC 0(0.0) | 99(13.2)   | 703(87.3) | NA* 0.016 | 0.08 |
|        |                       | Controls| G 79(4.1)  | T 1853(95.9) |            |            |      |      | 0(0.0)   | 79(8.2)    | 887(91.8) |        |     |
|        |                       |        |            |              |            |            |      |      |          |            |        |     |
| IFIH1  | rs1990760             | DM     | G 226(21.9)| T 808(78.1)  | 1.04(0.87–1.25) | 0.67 | 3.35 | GC 31(6.0) | 164(31.7) | 322(62.3) | 1.65 0.44 | 2.20 |
|        |                       | PM     | G 136(23.4)| T 446(76.6)  | 1.14(0.91–1.42) | 0.26 | 1.30 | GC 18(6.2) | 100(34.4) | 173(59.4) | 1.58 0.45 | 2.25 |
|        |                       | DM+PM  | G 362(22.4)| T 1254(77.6) | 1.07(0.92–1.26) | 0.38 | 1.90 | GC 49(6.1) | 264(32.6) | 495(61.3) | 2.05 0.36 | 1.80 |
|        |                       | Controls| G 410(21.2)| T 1526(78.8) |            |            |      |      | 44(4.5)  | 322(33.3) | 602(62.2) |        |     |
|        |                       |        |            |              |            |            |      |      |          |            |        |     |
|        | rs3747517             | DM     | G 338(33.0)| T 686(67.0)  | 0.98(0.83–1.15) | 0.78 | 3.90 | GA 52(10.2) | 234(45.7) | 226(44.1) | 0.61 0.74 | 3.70 |
|        |                       | PM     | G 198(34.5)| T 376(65.5)  | 1.05(0.86–1.27) | 0.66 | 3.30 | GA 30(10.4) | 138(48.1) | 119(41.5) | 1.31 0.52 | 2.60 |
|        |                       | DM+PM  | G 536(33.5)| T 1062(66.5) | 1.00(0.87–1.15) | 0.98 | 4.90 | GA 82(10.3) | 372(46.5) | 345(43.2) | 1.15 0.56 | 2.80 |
|        |                       | Controls| G 648(33.5)| T 1286(66.5) |            |            |      |      | 110(1.4) | 428(44.3) | 429(44.3) |        |     |
|        |                       |        |            |              |            |            |      |      |          |            |        |     |
| IRF5   | rs4728142             | DM     | G 179(17.2)| T 863(82.8)  | 1.30(1.05–1.59) | 0.01 | 0.05 | AA 8(1.5)  | 163(31.3) | 350(67.2) | 9.56 8.4×10⁻³ | 0.02 |
|        |                       | PM     | G 98(17.3) | T 470(82.7)  | 1.30(1.01–1.68) | 0.04 | 0.20 | AA 9(1.8)  | 80(16.2)  | 195(68.0) | 4.30 0.12 | 0.60 |
|        |                       | DM+PM  | G 277(17.2)| T 133(82.8)  | 1.30(1.08–1.56) | 5.2×10⁻³ | 0.026 | AA 17(2.1) | 243(30.2) | 545(67.7) | 9.30 9.6×10⁻³ | 0.048 |
|        |                       | Controls| G 267(13.8)| T 166(86.2)  |            |            |      |      | 18(1.9)  | 231(23.9) | 718(74.2) |        |     |

PM: polymyositis; DM: dermatomyositis; OR: odds ratio; CI: confidence interval; χ²: Chi-square test; P: P value corrected by Bonferroni method; NA: not available; *: the P value of genotypic analysis was calculated under the logistic regression analysis. **: This research's result demonstrated that rs5029939 GG genotype in PM patients was 0. We failed to calculate its genotypic frequency.

doi:10.1371/journal.pone.0110044.t002
Table 3. Analysis of the five SNPs based on three genetic models.

| Gene   | SNPs   | Group | Additive model | Dominant model | Recessive model |
|--------|--------|-------|----------------|----------------|-----------------|
|        |        |       | \( P_c \)     | \( OR (95\%CI) \) | \( P_c \)     | \( OR (95\%CI) \) | \( P_c \) | \( OR (95\%CI) \) |
| TNFAIP3| rs2230926 | DM    | 0.16           | 1.44(1.03–2.02) | 0.32           | 1.40(0.98–2.00) | 0.35     | 7.56(0.84–67.8)   |
|        |        | PM    | 4.8 \times 10^{-3} | 1.89(1.30–2.77) | 8.2 \times 10^{-3} | 1.89(1.27–2.80) | 0.60     | 6.73(0.61–74.5)   |
|        |        | DM+PM | 9.1 \times 10^{-3} | 1.59(1.19–2.13) | 0.02           | 1.57(1.15–2.13) | 0.33     | 7.26(0.87–60.5)   |
|        | rs5029939 | DM    | 0.10           | 1.51(1.07–2.12) | 0.20           | 1.45(1.02–2.07) | NA       | NA               |
|        |        | PM    | 4.7 \times 10^{-3} | 2.00(1.31–2.91) | 4.7 \times 10^{-3} | 2.00(1.31–2.91) | NA       | NA               |
|        |        | DM+PM | 5.7 \times 10^{-3} | 1.66(1.22–2.24) | 0.01           | 1.63(1.20–2.22) | NA       | NA               |
| IFIH1  | rs1990760 | DM    | 3.36           | 1.04(0.87–1.25) | 4.86           | 1.00(0.80–1.24) | 1.13     | 1.34(0.84–2.15)   |
|        |        | PM    | 1.32           | 1.13(0.91–1.41) | 2.00           | 1.12(0.86–1.47) | 1.29     | 1.39(0.79–2.44)   |
|        |        | DM+PM | 1.93           | 1.07(0.92–1.26) | 3.44           | 1.04(0.86–1.26) | 0.77     | 1.36(0.89–2.06)   |
|        | rs3747517 | DM    | 3.92           | 0.98(0.83–1.15) | 4.67           | 1.01(0.81–1.25) | 2.38     | 0.88(0.62–1.25)   |
|        |        | PM    | 3.29           | 1.05(0.86–1.27) | 1.92           | 1.13(0.86–1.47) | 3.32     | 0.91(0.59–1.39)   |
| IRF5   | rs4728142 | DM    | 4.91           | 1.00(0.87–1.15) | 3.09           | 1.05(0.87–1.27) | 2.27     | 0.89(0.66–1.21)   |
|        |        | PM    | 0.06           | 1.31(1.06–1.62) | 0.02           | 1.41(1.12–1.78) | 3.24     | 0.82(0.36–1.90)   |
|        |        | DM+PM | 0.21           | 1.30(1.01–1.68) | 0.31           | 1.32(0.99–1.76) | 0.94     | 1.73(0.77–3.88)   |
|        |        |       | 0.02           | 1.31(1.09–1.58) | 0.01           | 1.38(1.12–1.69) | 3.53     | 1.14(0.58–2.22)   |

PM: polymyositis; DM: dermatomyositis; OR odds ratio; CI confidence interval; \( P_c \): \( P \) value corrected by Bonferroni method; NA: not available.

\(^\dagger\): The GG genotype frequencies of rs5029939 were too low to carry out recessive genetic model analysis.

doi:10.1371/journal.pone.0110044.t003
### Table 4. Association between the five SNPs and PM/DM with ILD.

| Disease | Group   | \( P_{c} \) | OR (95%CI) | \( P_{c} \) | OR (95%CI) | \( P_{c} \) | OR (95%CI) | \( P_{c} \) | OR (95%CI) | \( P_{c} \) | OR (95%CI) |
|---------|---------|--------------|------------|--------------|------------|--------------|------------|--------------|------------|--------------|------------|------------|
| DM      | P vs. N | 3.14         | 0.88(0.53–1.47) | 4.11         | 1.06(0.63–1.79) | 0.53         | 0.78(0.58–1.05) | 4.00         | 1.04(0.80–1.35) | 2.51         | 0.90(0.65–1.24) |
|         | P vs. C | 0.64         | 1.37(0.91–2.07)  | 1.77         | 1.54(1.03–2.31) | 2.76         | 0.93(0.74–1.17)  | 4.70         | 0.99(0.82–1.21)  | 0.51         | 1.23(0.96–1.59)  |
|         | N vs. C | 0.22         | 1.56(1.01–2.41)  | 0.53         | 1.45(0.92–2.29) | 0.79         | 1.19(0.93–1.52)  | 3.55         | 0.96(0.77–1.20)  | 0.15         | 1.38(1.05–1.81)  |
| PM      | P vs. N | 3.86         | 1.10(0.59–2.03)  | 4.65         | 1.03(0.55–1.92) | 3.30         | 0.92(0.62–1.35)  | 3.82         | 0.95(0.67–1.34)  | 2.64         | 1.15(0.74–1.79)  |
|         | P vs. C | 0.016        | 1.96(1.24–3.10)  | 0.03         | 1.90(1.19–3.05) | 2.73         | 1.09(0.82–1.45)  | 4.37         | 1.02(0.79–1.31)  | 0.21         | 1.39(1.01–1.90)  |
|         | N vs. C | 0.13         | 1.79(1.07–3.00)  | 0.09         | 1.85(1.10–3.11) | 1.30         | 1.19(0.88–1.61)  | 3.00         | 1.08(0.82–1.41)  | 1.57         | 1.20(0.84–1.72)  |
| DM+PM   | P vs. N | 4.22         | 0.96(0.65–1.42)  | 4.16         | 1.04(0.70–1.56) | 0.60         | 0.83(0.66–1.05)  | 4.95         | 1.00(0.81–1.24)  | 4.35         | 0.98(0.75–1.27)  |
|         | P vs. C | 0.04         | 1.58(1.12–2.22)  | 0.02         | 1.67(1.18–2.36) | 4.5          | 0.99(0.81–2.00)  | 4.90         | 1.00(0.85–1.19)  | 0.11         | 1.29(1.04–1.60)  |
|         | N vs. C | 0.04         | 1.64(1.14–2.37)  | 0.07         | 1.60(1.10–2.33) | 0.47         | 1.19(0.97–1.46)  | 4.96         | 1.00(0.83–1.20)  | 0.11         | 1.31(1.04–1.66)  |

DM: deramatomyositis; PM: polymyositis; ILD: interstitial lung disease; Group P: patients with ILD; Group N: patients without ILD; Group C: Healthy controls; \( P_{c} \): \( P \) value corrected by Bonferroni method. Group P (DM: n = 297; PM: n = 166; DM+PM: n = 463); Group N (DM: n = 233; PM: n = 132; DM+PM: n = 365); Group C (n = 968).

doi:10.1371/journal.pone.0110044.t004

### Table 5. Haplotype analysis of TNFAIP3 SNPs between patients and controls.

| Haplotypes | Groups | rs2230926 | rs5029939 | Total of frequency | Case | Control | \( \chi^2 \) | \( P_{c} \) |
|------------|--------|-----------|-----------|--------------------|------|---------|------------|----------|
| DM         | C      | T         | 0.95      | 0.94               | 0.96 | 6.73    | 0.04       |          |
|            | G      | G         | 0.05      | 0.06               | 0.04 | 5.57    | 0.09       |          |
| PM         | C      | T         | 0.95      | 0.92               | 0.96 | 12.0    | 2.5 \times 10^{-3} |        |
|            | G      | G         | 0.05      | 0.08               | 0.04 | 10.2    | 7 \times 10^{-3}   |        |
| DM+PM      | C      | T         | 0.95      | 0.93               | 0.96 | 12.3    | 2.0 \times 10^{-3} |        |
|            | G      | G         | 0.05      | 0.07               | 0.04 | 10.4    | 6.5 \times 10^{-3} |        |

PM: polymyositis; DM: deramatomyositis; \( \chi^2 \): Chi-square test; \( P_{c} \): \( P \) value corrected by Bonferroni method.

doi:10.1371/journal.pone.0110044.t005
rs2230926 and rs5029939 (\(r^2 = 1\)). The CT haplotype (rs2230926 C–rs5029939 T) had a lower frequency between DM, PM or PM/DM patients and controls (\(P_c = 0.04\), \(P_c = 2.5 \times 10^{-3}\) and \(P_c = 2.0 \times 10^{-3}\), respectively)(Table 5).

Figure 1. Linkage disequilibrium (LD) analysis of the SNPs in the TNFAIP3 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 two SNPs (rs2230926 and rs5029939) in TNFAIP3 reside in an LD block. (A): The data from HapMap CHB. B: The data analysis between DM patients and healthy controls from our study. C: The data analysis between PM patients and healthy controls from our study. D: The data analysis between PM/DM patients and healthy controls from our study.

doi:10.1371/journal.pone.0110044.g001

Discussion

The sample size of present study was the largest candidate gene association study executed in PM/DM to date and the first one to investigate the association of TNFAIP3, IFIH1, and IRS5 polymorphisms with PM/DM in Chinese Han population [5–13]. We intend to examine the genetic contribution of TNFAIP3,
IFIH1, and IRF5 to PM/DM based upon the postulated roles of each of these genes' products in innate and cell-mediated immunity in PM/DM and their described associations with autoimmune diseases. Significantly, our study confirmed TNFAIP3 and IRF5 gene polymorphisms were associated with PM/DM patients or these patients with ILD, and indicated that TNFAIP3 and IRF5 might be the susceptibility gene for PM/DM patients in Chinese Han population. The results were consistent with the previous findings undertaken on other auto-inflammation diseases with regard to TNFAIP3 and IRF5 gene polymorphisms [15–17,25–27].

The TNFAIP3 gene, encoded ubiquitin-modifying enzyme known as A20, inhibits the activation of NF-κB signaling pathways, including these TNF and Toll-like receptors [42]. The deficiencies of A20 expression are related with the development of various human autoimmune disorders [43]. The immune responses in A20-deficient mice present severe multi-organ inflammation, damage to joints, and finally develop autoimmunity [44]. Furthermore, the mice, which lacked A20 in myeloid cells spontaneously, finally turn into RA with many features such as severe destructive polyarthritis [45]. In addition, Deficiency of A20 in B cells results in inflammation and leads to autoimmune response in old mice [46]. Previously, plenty of GWAS and candidate gene association studies suggested TNFAIP3 gene loci rs2230926 and rs5029939 were associated with diverse rheumatic diseases [15–17]. Studies denoted that DM could be overlapped with SSC [47], SLE [40] and other connective-tissue disease. In the current study of the Han Chinese population, TNFAIP3 SNPs (rs2230926 and rs5029939) illustrated significant association with PM patients or PM/DM patients and these patients with ILD. Thus, rs2230926 and rs5029939 may in fact play a dominant role in the pathogenesis of multiple autoimmune diseases as well as to PM/DM. It is also worthwhile to note that in the present study, the GG genotype frequencies of rs2230926 and rs5029939 were too low to carry out genotypic analysis and recessive genetic model analysis. The P value of genotypic analysis was calculated under the logistic regression analysis. Future studies about PM/DM patients using larger sample sizes should be performed to confirm these outcomes.

The IFIH1 gene is a member of the retinoic acid-inducible gene 1-like helicase (RLH) family [49–50], and IFIH1 gene encodes a viral RNA-activated apoptosis protein, which is an early IFN beta signaling protein. Walsh et al. [51] demonstrated that IFNβ-inducible genes (such as IFIH1 gene) were the greatest highly overexpressed genes in patients with active DM and patients with PM, but not in healthy controls. And the up-regulation of the IFN protein signature had increased additional markers of disease activity and insight into the pathogenesis of PM/DM. Until now, a large number of studies indicated IFIH1 polymorphisms showed susceptibility to multiple autoimmune diseases [19–22]. Recently, an investigation, regarding IFIH1 rs1990760 associated with susceptibility to SLE and PM/DM, was performed in the Japanese population, which suggested IFIH1 rs1990760 polymorphism was not significantly associated with PM/DM as a whole in this study, but only showed the AA genotype tended to be found with higher frequency in the PM with ILD subset. Similarly, our current study carried out in the Chinese population didn’t manifest the positive associations between IFIH1 polymorphisms and PM/DM patients or these patients with ILD. The SNPs (rs1990760 and rs3747517) in IFIH1 gene chose in our study were those with the strongest associations with other autoimmune disease in the Chinese Han population. As the SNPs in our study did not illustrate positive associations with PM/DM, future studies might evaluate whether other SNPs in IFIH1 gene are associated with susceptibility to PM/DM.

The IRF5 gene is a part of the transcription factor IRF family, which contains nine transcription factors. This gene encodes IRF protein with diverse roles, including virus-mediated activation of interferon, and modulation of cell growth, differentiation, apoptosis, and immune system activity. When viruses infect, expression of IRF5 is up-regulated by IFN-α, and subsequently IRF5 up-regulates IFN-inducible genes, comprising pro-inflammatory cytokines such as IL-10, and also those participated in apoptosis and the early immune response [52]. Therefore, this up-regulation of important molecules in the IFN signaling pathway may have prominent functional influence on the pathogenesis of autoimmune diseases. Hence, numerous of studies found IRF5 gene polymorphisms were associated with susceptibility to multiple autoimmune disorders [25–27]. Significantly, this present candidate gene association studies regarding IRF5 polymorphism associated with PM/DM risk demonstrated positive results. And the consequences were consistent with the previous findings undertaken on other auto-inflammation diseases. Although we excluded rs729302 because of its departure from HWE in the control group, the correlation of this SNP to PM/DM was seen without consideration of HWE (data not shown), implying a possible association between rs729302 and PM/DM in Han Chinese population.

Because IIMs are a group of rare autoimmune disease, difficulties always were encountered in previous genetic association studies when investigator recruited an adequate number of patients for analysis in studies that examined SNPs with an appropriate sample size. Given the above limitations, our study firstly enrolled the largest number of PM/DM patients in Chinese Han population, who fulfilled the international guidelines [31–32]. Therefore, the present investigation had sufficient statistical power (more than 90%) to examine moderate or even marginal associations. It is a remarkable fact that our study found significantly positive associations between TNFAIP3 (rs2230926 and rs5029939), IRF5 (rs4720142) gene polymorphisms and PM/DM or these patients with ILD. None of these SNPs was previously reported to be associated with PM/DM or these patients with ILD. Therefore, these SNPs may play a potential role in the pathogenesis of PM/DM or these patients with ILD. In addition, our study failed to analyze the potential association of these genetic variants with some special clinical subtypes of PM/DM in this population, such as serological phenotypes (autoantibody profiles).

In summary, our present study was the first investigation to indicate that TNFAIP3 and IRF5 might be the susceptibility gene for PM/DM patients in Chinese Han population. Although the present study is the largest candidate gene association study performed to date for PM/DM, it is still limited and more research is required to understand the associations of TNFAIP3, IFIH1, and IRF5 with PM/DM in different ethnic populations. In the Chinese population, it isn’t been reported about GWAS on PM/DM, in order to better understand the pathogenesis of PM/DM, we are looking forward to conduct the GWAS on PM/DM in the Chinese population.

**Supporting Information**

**Table S1** The detailed information of SNPs in this study.
Acknowledgments

We’d like to give our sincere appreciation and thanks to all the patients with PM/DM, who made this study possible, to Chunwei Gao for the useful suggestion he offered, to Yang Du for the expertise and technical assistance.

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Author Contributions

Conceived and designed the experiments: SC QW YZL. Performed the experiments: SC QW. Analyzed the data: SC QW. Contributed reagents/materials/analysis tools: YL PL WJZ FS CJW CYW CWD FGZ. Wrote the paper: SC QW. Performed the PLINK v1.07 software (Shaun Purcell, Boston, USA). SC QW. Performed the Genetic Power Calculator and Haplview software v4.2: QW YL.
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