Inflammation-Related Gene Polymorphisms Associated With Primary Immune Thrombocytopenia

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INTRODUCTION

Primary immune thrombocytopenia (ITP) is an acquired autoimmune disease characterized by a reduced platelet count and an increased risk of bleeding (1). The pathogenesis of ITP includes enhanced platelet clearance and impaired platelet production, which is related to T cell-mediated effects, B cell-mediated effects, tolerance checkpoint defects, and more (2, 3). Although immense research has improved our understanding of ITP, the pathogenesis remains unclear. Here, we investigated the involvement of 25 single-nucleotide polymorphisms (SNPs) of the inflammation-related genes, including CD24, CD226, FCRL3, IL2, IRF5, ITGAM, NLRP3, CARD8, PTPN22, SH2B2, STAT4, TNFAIP3, and TRAF1, in the pathogenesis and treatment response of ITP. We recruited 312 ITP inpatients and 154 healthy participants in this case–control study. Inflammation-related SNP genotyping was performed on the Sequenom MassARRAY iPLEX platform. The expression of TNFAIP3 mRNA was determined by quantitative real-time RT-PCR.
Several therapeutic agents have been recommended for the management of ITP, including corticosteroids, immunoglobulin, and corticosteroid-sparing agents such as intravenous immunoglobulin (IVIG) and rituximab (18–20). Particularly, corticosteroids remain the first-line therapeutic option for ITP (20). However, up to 30–40% of patients may fail to respond to corticosteroids (21, 22). For these patients, therapeutic options include splenectomy, azathioprine, and anti-thymocyte globulin (22). Alternatively, a therapeutic trial with different platelet-enhancing agents has been recommended for severe ITP patients who fail splenectomy (21). Currently, the management of severe ITP is individualized based on patient’s response and side effects (21). In other cases, the therapeutic intervention with different platelet-enhancing agents includes rituximab, IVIG, Rituximab, or rituximab plus IVIG (21).

In this study, the outcome of corticosteroid treatment was assessed with the primary and secondary endpoints. The primary endpoint was defined as a platelet count of 100,000/μL or higher and a minimum twofold increase in platelet count compared to the baseline platelet count. The secondary endpoints included the platelet count and the diagnostic criteria for ITP. The study criteria were based on the guidelines of the International Working Group for ITP (23). The study included patients who were diagnosed with ITP according to the criteria of the International Working Group for ITP (23). The study was approved by the institutional review board of the hospital, and written consent was obtained from all participants.
For the control group, 205 healthy participants were enrolled. Controls were randomly selected from healthy volunteers with no symptoms of ITP and no history of other autoimmune diseases. All participants were Han Chinese and no genetic associations were found between any participants.

The study was approved by the Medical Ethical Committee of Qilu Hospital, Shandong University. Written informed consent was obtained from each participant in accordance with the Declaration of Helsinki.

DNA Extraction and Genotyping
Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) that had been isolated from whole blood samples (5 mL) from participants by a standard protocol. DNA concentration and purity were assessed at 260/280 absorbance by a NanoDrop spectrophotometer. The DNA extraction was stored at −20°C until genotyping. Inflammation-related SNPs (summarized in Table 1) genotyping was performed on a MassArray system (Sequenom iPLEXassay, BGI Tech., Beijing, China), which is based on a multiplex PCR reaction, a locus-specific single-base extension reaction, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

Statistical Analysis
The p value of the Hardy–Weinberg equilibrium (HWE) was calculated using the calculator available at the Helmholtz Center Munich website. In addition to allelic frequencies, we analyzed genotypic frequencies under three genetic models, specifically, the codominant, dominant, and recessive models. Associations between the SNPs and ITP susceptibility, severity, corticosteroid sensitivity, and refractoriness were calculated by a chi-squared (χ²) test or a Fisher’s exact test. Univariate and multivariate binary logistic regression analyses were used to analyze adjusted p values and odds ratios (ORs) with a corresponding 95% confidence interval (95% CI). A two-tailed p < 0.05 (or adjusted value by Bonferroni multiple testing) was considered statistically significant. All statistical analyses were performed using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA).

| TABLE 1 | Selected genes and SNPs. |
| Genes | SNPs |
| CD24 | rs8734 |
| FCRL3 | rs11264799, rs7528884, rs945635, rs37679159 |
| CD22B | rs762361 |
| IL2 | rs6822844 |
| IRF5 | rs2004640, rs2280714, rs10954213 |
| ITGAM | rs1143679 |
| NLRP3 | rs35829419, rs4353135, rs10754558 |
| CARD8 | rs2043211 |
| PTPN22 | rs33996649, rs1310182 |
| SH2B3 | rs3184504 |
| STAT4 | rs7574866, rs10181656 |
| TNFAIP3 | rs6820220, rs10499194, rs2230926, rs5029939 |
| TRAF1 | rs10818488 |

SNP, single-nucleotide polymorphism.

RNA Extraction and Real-time RT-PCR of TNFAIP3
Total RNA of PBMCs was isolated by TRIzol reagent (Invitrogen). RNA was converted into cDNA using the PrimeScript RT reagent kit (Perfect Real Time; Takara) according to the manufacturer’s instructions. Multiplex real-time RT-PCR was performed for TNFAIP3 and the endogenous control β-actin on an ABI PRISM_7500 Sequence Detection System (Applied Biosystems) using SYBR Green (Toyobo) as a double-strand DNA-specific binding dye. The primers for all mRNA assays were intron spanning. The PCR reactions were cycled 40 times after initial denaturation (95°C, 10 min) at 95°C for 15 s and at 60°C for 30 s. The primers for TNFAIP3 and β-actin are as follows: TNFAIP3 forward: GTGTATTTTGGGACTCCAGA, TNFAIP3 reverse: ACTTCTGGCAGTATCCTTTCA; β-actin forward: CACCAAC TGGGACGACAT; β-actin reverse: GCACAGCCTGGATAGCA AC. We used the comparative threshold cycle (Ct) method for relative quantification of TNFAIP3 mRNA according to relative expression software tool (Michael) (42). The amplification efficiency between the target (TNFAIP3) and the reference control (β-actin) were compared to use the delta G (ΔΔCt) calculation.

RESULTS

Study Population
Demographic and clinical characteristics of controls and ITP patients are summarized in Table 2. All inflammation-related SNPs in the control group were in accordance with HWE. No significant deviations were observed after Bonferroni multiple correction (p > 0.002, Table S1 in Supplementary Material).

Association between Inflammation-Related SNPs and ITP Susceptibility
Four genetic models were used to analyze the association between the 25 inflammation-related SNPs and ITP. We analyzed the relationship between every single locus and the susceptibility to ITP by a chi-squared (χ²) test or a Fisher’s exact test (Table S1 in Supplementary Material). Preliminary screening showed that allelic frequencies of rs8734 in CD24 and genotypic frequencies

| TABLE 2 | Demographic and clinical characteristics. |
| Controls | ITP patients |
| No. | 205 | 312 |
| Age, mean ± SD | 45.72 ± 13.41 | 39.96 ± 13.69 |
| Gender (M/F) | 75/130 | 116/196 |
| ITP severity, n (%) | | |
| Severe ITP | NA | 189 (60.6) |
| Non-severe ITP | NA | 123 (39.4) |
| Treatment, n (%) | | |
| No use of corticosteroid | NA | 50 (16.0) |
| Corticosteroid-sensitive | NA | 156 (50.0) |
| Corticosteroid-resistant | NA | 106 (34.0) |
| Refractory ITP | NA | 21 (6.7) |
| Non-refractory ITP | NA | 291 (93.3) |

M, male; F, female; NA, not applicable; ITP, immune thrombocytopenia.
of rs11264799 in FCRL3 under the codominant model were significantly associated with susceptibility to ITP ($p < 0.05$). In addition, both the allelic and genotypic frequencies of rs10499194 in TNFAIP3 under the codominant and dominant models were significantly associated with the susceptibility to ITP ($p < 0.05$). Among the above SNPs, only rs10499194 was associated with susceptibility of ITP after Bonferroni multiple correction.

Univariate logistic regression analysis revealed that for rs8734 in CD24, allele A in place of G was significantly associated with susceptibility to ITP after adjusting for age and gender ($p = 0.035$, Table 3). For rs11264799 in FCRL3, the CT rather than CC genotype was significantly associated with susceptibility to ITP under the codominant model ($p = 0.003$, Table 3). For rs10499194 in TNFAIP3, CT and CC/CT genotypes were both statistically significant compared to CC ($p = 0.001$ and $p = 0.000$, respectively, Table 3). rs10499194 allelic distribution also showed a statistically significant difference ($p = 0.003$; Table 3). Interestingly, all three inflammation-related polymorphisms demonstrated a protective effect. Among these polymorphisms, only rs10499194 presented a significant association with susceptibility to ITP with univariate logistic regression analysis after Bonferroni multiple correction.

Next, we performed a multivariate logistic regression analysis under the codominant model. We found that the heterozygous genotypes of FCRL3 rs11264799 and TNFAIP3 rs10499194 significantly decreased the risk of ITP compared with homozygous major alleles ($p = 0.029$ and $p = 0.001$, respectively, Table 4). When we analyzed the combined influence of allelic distribution of both CD24 rs52812045 and TNFAIP3 rs10499194, we found a statistical difference in TNFAIP3 rs10499194 between ITP patients and controls ($p = 0.006$, Table 5).

### Association between Inflammation-Related SNPs and ITP Severity

We next evaluated the association of the inflammation-related SNPs with ITP disease severity. CARD8 rs2043211 and TRAF1 rs10818488 genotypes were statistically different between severe and non-severe ITP patients according to chi-squared analyses ($p < 0.05$, Table S2 in Supplementary Material). However, neither difference persisted after Bonferroni multiple correction. CARD8 rs2043211 lost the statistical difference after adjusting for age and gender factors by univariate binary logistic regression under dominant model (AA + AT vs. TT; OR = 0.589, 95% CI = 0.343–1.013, $p = 0.056$). After adjusting for age and gender, ITP patients carrying the AG genotype of TRAF1 rs10818488 showed a 1.713-fold increased risk of developing severe ITP compared with patients carrying major genotype GG under codominant model.

### Table 3 | Association between selected SNPs and ITP risk

| Gene   | SNP         | Model/allele | Genotype/allele | Controls Count | Controls % | ITP patients Count | ITP patients % | OR (95% CI) | Adjusted $p$ value |
|--------|-------------|--------------|-----------------|----------------|------------|---------------------|----------------|-------------|-------------------|
| CD24   | rs52812045  | Allele       | G               | 244            | 59.5       | 411                 | 65.9           | 1.000       | 0.035             |
|        |             |              | A               | 166            | 40.5       | 213                 | 34.1           | 0.754 (0.580–0.981) |
| FCRL3  | rs11264799  | Codominant   | CC              | 119            | 58.0       | 205                 | 65.7           | 1.000       | 0.003             |
|        |             |              | TT              | 3              | 1.5        | 11                  | 3.5            | 0.544 (0.678–9.547) |
|        |             |              | CT              | 83             | 40.5       | 96                  | 30.8           | 0.668 (0.458–0.975) |
| TNFAIP3| rs10499194  | Codominant   | CC              | 160            | 78.0       | 277                 | 88.8           | 1.000       | 0.000             |
|        |             |              | TT              | 1              | 0.5        | 2                   | 0.6            | 1.235 (0.106–14.385) |
|        |             |              | CT              | 44             | 21.5       | 33                  | 10.6           | 0.431 (0.262–0.711) |
|        |             | Dominant     | CC              | 160            | 78.0       | 292                 | 93.6           | 1.000       | 0.000             |
|        |             |              | TT/CT           | 45             | 22.0       | 20                  | 6.4            | 0.249 (0.141–0.440) |
|        |             | Allele       | C               | 364            | 88.8       | 587                 | 94.1           | 1.000       | 0.003             |
|        |             |              | T               | 46             | 11.2       | 37                  | 5.9            | 0.499 (0.315–0.791) |

SNP, single-nucleotide polymorphism; CI, confidence interval; OR, odds ratio; ITP, immune thrombocytopenia.

Adjusted $p$ value calculated with univariate logistic regression.

Bold highlights statistical significance ($p < 0.05$).

### Table 4 | Association between selected SNPs and ITP risk under codominant model

| Genes  | SNP         | Genotype | Controls Count | Controls % | ITP patients Count | ITP patients % | OR (95% CI) | Adjusted $p$ value |
|--------|-------------|----------|----------------|------------|---------------------|----------------|-------------|-------------------|
| FCRL3  | rs11264799  | CC       | 119            | 58.0       | 205                 | 65.7           | 1.000       | 0.141             |
|        |             | TT       | 3              | 1.5        | 11                  | 3.5            | 2.703 (0.719–10.161) |
|        |             | CT       | 83             | 40.5       | 96                  | 30.8           | 0.653 (0.446–0.958) |
| TNFAIP3| rs10499194  | CC       | 160            | 78.0       | 277                 | 88.8           | 1.000       | 0.029             |
|        |             | TT       | 1              | 0.5        | 2                   | 0.6            | 1.285 (1.014–15.869) |
|        |             | CT       | 44             | 21.5       | 33                  | 10.6           | 0.415 (0.250–0.689) |

SNP, single-nucleotide polymorphism; CI, confidence interval; OR, odds ratio; ITP, immune thrombocytopenia.

Adjusted $p$ value calculated with multivariate logistic regression.

Bold highlights statistical significance ($p < 0.05$).
(OR = 1.713, 95% CI = 1.008–2.911, p = 0.047). In summary, only the AG genotype of TRAF1 rs10818488 increased the risk of severe ITP among patients.

**Association between Inflammation-Related SNPs and Corticosteroid Sensitivity**

After studying associations with the pathogenesis of ITP, we explored the association between the inflammation-related polymorphisms and the treatment of ITP. Specifically, we studied corticosteroid sensitivity and refractoriness among patients with different genotypes or alleles. To study corticosteroid sensitivity, we divided the patients who received corticosteroid treatment into two groups, the corticosteroid-sensitive group (n = 156) and the corticosteroid-resistant group (n = 106).

We found statistically significant associations between inflammation-related polymorphisms and corticosteroid sensitivity. rs945635, rs7528684, and rs3761959 of FCRL3 and rs2004640 of IRF5 under the dominant model were significantly associated with corticosteroid-sensitivity (p < 0.05, Table S3 in Supplementary Material). rs4353135 of NLRP3 under both the codominant and recessive models showed significant association with corticosteroid sensitivity (p < 0.05, Table S3 in Supplementary Material). Allelic frequencies and genotypic frequencies under the dominant and recessive models of TNFAIP3 rs10499194 revealed statistically significant differences between the corticosteroid-sensitive and corticosteroid-resistant groups (p < 0.05, Table S3 in Supplementary Material). TNFAIP3 rs10499194 remained significant after Bonferroni multiple correction.

Allelic and genotypic frequencies were analyzed by logistic regression analysis. After univariate logistic regression analysis, compared with major allele homozygotes, minor allele homozygotes, and heterozygotes of FCRL3 rs945635, rs7528684, and rs3761959 and IRF rs2004640 were still significantly associated with corticosteroid-sensitivity under the dominant model after adjusting for age and gender (p = 0.029, p = 0.029, p = 0.029, p = 0.024, respectively, Table 6). Genotypic frequencies of NLRP3 rs4353135 were significantly different under the recessive model between the corticosteroid-sensitive and -resistant groups (p = 0.028, Table 6). Genotypic and allelic frequencies of TNFAIP3 rs10499194 were significantly different under the codominant and dominant models (all p = 0.001, Table 6). Importantly, the observed differences in TNFAIP3 rs10499194 were still statistically significant after Bonferroni multiple correction (p < 0.002).

Multivariate logistic regression showed significant associations between FCRL3 rs945635, rs7528684, and rs3761959, IRF rs2004640, and TNFAIP3 rs10499194 and corticosteroid sensitivity (p = 0.009, p = 0.009, p = 0.009, p = 0.012, and p = 0.000, respectively, Table 6). TNFAIP3 rs10499194 was significantly associated with corticosteroid-sensitivity under both univariate and multivariate logistic regression analyses after Bonferroni multiple correction (p < 0.002).

**Association between Inflammation-Related SNPs and ITP Refractoriness**

To study refractoriness, we divided the enrolled patients into two subgroups: those refractory to splenectomy treatment and those who responded to medical treatment. We analyzed the association between the inflammation-related SNPs and refractoriness of ITP using chi-squared tests for preliminary screening. The genotypic distribution of STAT4 rs7574869 was significantly associated with refractoriness under the codominant model (p < 0.05, Table S4 in Supplementary Material). No statistical differences were found between other SNPs and refractoriness (p > 0.05). However, the association between STAT4 rs7574869 and refractoriness was not statistically significant after Bonferroni multiple correction. When the GG genotype was used as reference, neither the TT nor GT genotypes were significantly associated with refractoriness after adjusting for the age and gender (p = 0.998 and p = 0.108, respectively).

**Association of TNFAIP3 rs10499194 Polymorphism and Expression Levels of TNFAIP3**

To determine whether TNFAIP3 rs10499194 was a protective factor for ITP, we further explored the effects of this polymorphism on TNFAIP3 expression. The number of ITP patients with TT genotype was small (n = 2) in this study, which may lead to an underpowered assessment. The expression of TNFAIP3 was assessed in 85 ITP patients (60 cases of CC, 2 cases of TT, and 23 cases of CT) by quantitative real-time RT-PCR. As shown in Figure 1A, patients with CT genotype showed higher levels of TNFAIP3 mRNA expression than those with CC genotype (p = 0.001). We found that TNFAIP3 expression was significantly higher in samples with pooled CT/TT genotype compared with the CC genotype (p = 0.001; Figure 1B). The result showed that CC genotype on TNFAIP3 rs10499194 was a protective factor for ITP.
TABLE 6 | Association between selected SNPs and corticosteroid-sensitivity of ITP patients.

| Gene       | SNP        | Model/allele     | Genotype/allele | Sensitive Count | Sensitive % | Resistant Count | Resistant % | OR (95% CI)* | p Value* | OR (95% CI)# | p Value# |
|------------|------------|------------------|-----------------|----------------|-------------|----------------|-------------|--------------|----------|--------------|----------|
| TNFAIP3    | rs10499194 | Codominant       | CC              | 128            | 82.1        | 104            | 98.1        | 1            |          | 0.999        |          |
|            |            |                  | TT              | 2              | 1.3         | 0              | 0.0         | -            |          |              |          |
|            |            |                  | CT              | 26             | 16.7        | 2              | 1.9         | 0.092 (0.021–0.398) | 0.001    |              |          |
|            |            | Dominant         | CC              | 128            | 82.1        | 104            | 98.1        | 1            |          | 1.000        |          |
|            |            |                  | TT/CT           | 28             | 17.9        | 2              | 1.9         | 0.086 (0.020–0.369) | 0.001    | 0.069 (0.016–0.302) | 0.000    |
|            |            | Allele           | C               | 282            | 90.4        | 210            | 99.1        | 1.000        |          |              |          |
| IRF5       | rs2004640  | Dominant         | GG              | 96             | 61.5        | 51             | 48.1        | 1.000        |          | 1.000        |          |
|            |            |                  | TT/CT           | 60             | 38.5        | 55             | 51.9        | 1.793 (1.079–2.980) | 0.024    | 1.996 (1.162–3.429) | 0.012    |
| FCRL3      | rs3761959  | Dominant         | CC              | 65             | 41.7        | 30             | 28.3        | 1.000        |          | 1.000        |          |
|            |            |                  | TT/CT           | 91             | 58.3        | 76             | 71.7        | 1.807 (1.062–3.076) | 0.029    | 2.107 (1.208–3.674) | 0.009    |
| FCRL3      | rs7528684  | Dominant         | AA              | 65             | 41.7        | 30             | 28.3        | 1.000        |          | 1.000        |          |
|            |            |                  | GG/AG           | 58             | 34.7        | 18             | 30.8        | 1.807 (1.062–3.076) | 0.029    | 2.107 (1.208–3.674) | 0.009    |
| FCRL3      | rs9456335  | Dominant         | CC              | 65             | 41.7        | 30             | 28.3        | 1.000        |          | 1.000        |          |
|            |            |                  | GG/GG           | 91             | 58.3        | 76             | 71.7        | 1.807 (1.062–3.076) | 0.029    | 2.107 (1.208–3.674) | 0.009    |
| NLRP3      | rs4353135  | Codominant       | TT              | 49             | 31.4        | 36             | 34.0        | 1.000        |          |              |          |
|            |            |                  | GG              | 24             | 15.4        | 29             | 27.4        | 1.599 (0.798–3.205) | 0.186    |              |          |
|            |            |                  | GT              | 83             | 53.2        | 41             | 38.7        | 0.684 (0.385–1.212) | 0.193    |              |          |
|            |            | Recessive        | TT/CT           | 132            | 84.6        | 77             | 72.6        | 1.000        |          |              |          |
|            |            |                  | GG              | 24             | 15.4        | 29             | 27.4        | 1.991 (1.077–3.681) | 0.028    |              |          |

SNP, single-nucleotide polymorphism; CI, confidence interval; OR, odds ratio; ITP, immune thrombocytopenia.

*Calculated by univariate logistic regression.

#Calculated by multivariate logistic regression analysis under dominant model.

Bold highlights statistical significance (p < 0.05).

FIGURE 1 | (A) Expression of TNFAIP3 mRNA in ITP patients with the CC, CT and TT genotypes. (B) Expression of TNFAIP3 mRNA in ITP patients with the CC and pooled CT/TT genotypes.

DISCUSSION

Immune thrombocytopenia is an autoimmune and inflammatory disease characterized by immune-mediated platelet destruction. Although ITP has been associated with inflammation, only a few studies have identified the association between inflammation-related SNPs and the pathogenesis of ITP (43). By contrast, inflammation-related gene polymorphisms have been associated with multiple autoimmune diseases.

In this study, we assessed the associations between 25 inflammation-related SNPs and the occurrence and treatment of ITP in the Chinese Han population. Of these SNPs, the frequencies of the homozygous minor allele of FCRL3 rs11264799 and TNFAIP3 rs10499194 were significantly decreased in ITP patients. The A allele of CD24 rs8734 was associated with a decreased risk of ITP. In addition, compared with the CC genotype, individuals carrying pooled TT/CT genotypes of TNFAIP3 rs10499194 had a decreased risk of ITP under the dominant model. These three SNPs were all protective factors.

Our findings contrast evidence found regarding other autoimmune diseases (26, 44–48). For example, in the Spanish population, no association was found between FCRL3 rs11264799 and MS (44). Interestingly, however, the C allele of FCRL3 rs7528684 was a protective factor for MS. This suggests that FCRL3 polymorphisms may protect individuals from autoimmune diseases, which is somewhat consistent with our findings. In the Israeli
population, individuals with CD24 rs8734 polymorphisms had an increased risk of IBD, ulcerative colitis, and Crohn’s disease (26). In the Chinese population, a significant association was not found between TNFAIP3 rs10499194 and SLE (45); however, carriers of the CT genotype and combined TT/CT genotype had an increased risk for RA in the Chinese Han population (46). A recent meta-analysis revealed that there was no association between TNFAIP3 rs10499194 and RA in Europeans, but a significant association in Asians (OR = 1.254, 95% CI = 1.101–1.429, p = 6.7 × 10−4) (48). The discrepancies between our results and previous research may be attributed to differences in the populations and autoimmune diseases studied.

When it comes to TNFAIP3 rs10499194, there were several researches identified that it was significantly associated with the susceptibility of autoimmune diseases. Strong associations were observed between TNFAIP3 rs10499194 and juvenile idiopathic arthritis (OR = 0.74, 95% CI = 0.61–0.91, p < 0.004) (49). Prahalad et al demonstrated that TNFAIP3 rs10499194 had a significant protective effect against childhood onset RA (OR = 0.60, 95% CI = 0.44–0.83, p = 0.002) (50). Consistent with our findings, TNFAIP3 rs2230926 and rs5029939 were significantly different between chronic ITP and control groups (43), suggesting that TNFAIP3 polymorphisms may affect the susceptibility to ITP. We found that TNFAIP3 rs10499194 was significantly associated with the susceptibility of ITP even after Bonferroni multiple correction. Therefore, TNFAIP3 rs10499194 may be an important susceptibility-related SNP for ITP.

To investigate whether TNFAIP3 rs10499194 was a functional polymorphism, quantitative real-time RT-PCR was performed to evaluate TNFAIP3 expression. The data showed that individuals with CT genotype on TNFAIP3 rs10499194 locus showed higher levels of TNFAIP3 mRNA expression compared with the CC genotype, which might play a role in the susceptibility of ITP. It is widely accepted that TNFAIP3 is a deubiquitinating protein which can deregulate pro-inflammatory signal pathways including NF-kB- and IRF3-dependent gene expression by deubiquitinating specific signaling molecules (51, 52). Some researchers revealed that TNFAIP3 was a central gatekeeper in inflammation and immunity (53). A recent study demonstrated that lack of TNFAIP3 in B cells resulted in overexpression of pro-inflammatory cytokines, which caused inflammation and autoimmunity in aged mice (54). TNFAIP3-deficient B cells displayed enhanced proliferation and development of autoantibodies (55). In our functional analysis, individuals with CT genotype on TNFAIP3 rs10499194 locus showed higher levels of TNFAIP3 mRNA expression compared with the wild genotype CC, which is in agreement with our genotyping analysis that CT genotype is a protective factor for ITP. However, the mechanism of polymorphism on TNFAIP3 expression awaits further investigations.

Furthermore, we found that the AG genotype of TRAF1 rs10818488 was significantly associated with increased risk of severe ITP after adjusting for age and gender. These novel findings will be studied further in order to understand the molecular mechanism of these genetic polymorphisms.

In addition to the pathogenesis of ITP, the mechanism of corticosteroid resistance remains poorly understood. In acute lymphoblastic leukemia, the NLRP3-CASP1 inflammasome induced glucocorticoid resistance (56). Overexpression of CASP1 may promote cleavage of the glucocorticoid receptor, which decreased glucocorticoid sensitivity. We identified that NLRP3 rs4353135 was significantly associated with increased risk of corticosteroid resistance of ITP under the recessive model; however, this observation was not significant after Bonferroni multiple correction. Thus, we propose that the NLRP3 rs4353135 polymorphism may enhance expression of the NLRP3-CASP1 inflammasome and lead to corticosteroid resistance. Future studies will examine this hypothesis.

Importantly, TNFAIP3 rs10499194 was associated with corticosteroid-sensitivity after Bonferroni correction. Future work will examine the potential of TNFAIP3 rs10499194 as a biomarker for corticosteroid sensitivity.

There were some limitations to our study. On the one hand, the participants enrolled in our study were solely from Chinese Han population. Larger trials investigating multi-racial populations are needed to examine whether the associations between the inflammation-related SNPs and ITP exist in the general population. Second, as mentioned earlier, the biological functions and signaling pathways of these SNPs are not yet known and future research with global collaborations are required. Third, there was a potential selection bias in our study. Only inpatients were recruited in our study, as the corticosteroid-sensitivity and refractoriness of outpatients were difficult to follow-up in our hospital. The inpatients have more severe thrombocytopenia and bleeding symptoms. The bleeding symptoms may include gastrointestinal hemorrhage, extensive skin and mucosal hemorrhage, or intracranial hemorrhage. Besides, the 25 susceptibility loci, we tested were selected basing on the previous reported loci associated with other autoimmune diseases. The candidate gene approach cannot cover all susceptible loci, which may lead to the selection bias.

**CONCLUSION**

Our investigation of SNPs and ITP provides interesting results. We found that inflammation-related SNPs, especially TNFAIP3 rs10499194, may be genetic risk factors associated with the development and treatment of ITP. Our findings may lead to clinicians screening for these SNPs in order to predict the prognosis and guide the treatment of ITP.

**STATEMENT OF PRIOR PRESENTATION**

Part of this study was presented as a poster with a title of “Inflammation-related Gene Polymorphisms Associated with Susceptibility to Primary Immune Thrombocytopenia” (Abstract Code: 3737) and won ASH Abstract Achievement Award at the 58th ASH Annual Meeting and Exposition in San Diego, CA, USA, December 3–6, 2016.

**ETHICS STATEMENT**

This study was carried out in accordance with the recommendations of Medical Ethical Committee of Qilu Hospital, Shandong University with written informed consent from all subjects. All
subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Medical Ethical Committee of Qilu Hospital, Shandong University.

AUTHOR CONTRIBUTIONS

JP, MH, and X-hZ designed research, analyzed data, and wrote the paper; JL performed research, analyzed data, and wrote the paper; SM, LS, CM, and CG performed research and analyzed data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fimmu.2017.00744/full#supplementary-material.

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**Conflict of Interest Statement**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. 

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