Research Article

Association Study of the Caspase Gene Family and Psoriasis Vulgaris Susceptibility in Northeastern China

Xinyu Yao,1 Siyu Hao,2 and Pei Yu2

1Department of Dermatology, Peking University First Hospital, Beijing, 100034, China
2Department of Dermatology, The Second Affiliated Hospital of Harbin Medical University, Harbin, 150081, China

Correspondence should be addressed to Pei Yu; fenglinxing@163.com

Received 31 August 2018; Revised 27 December 2018; Accepted 31 January 2019; Published 17 February 2019

Academic Editor: Rachid Tazi-Ahnini

Copyright © 2019 Xinyu Yao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Background. Abnormal apoptosis of keratinocytes is one of the pathological changes of psoriasis. Caspases (CASP) are the central engines of apoptosis. Studies to date have shown that some SNPs alter the expression of related genes and lead to changes in disease risk. However, no studies have investigated the associations between gene polymorphisms and the risk of psoriasis in Han population in northeast China. Therefore, we conducted a case-control study to explore this question in Han population of northeastern China.

Methods. 540 patients with PsV and 612 healthy age- and sex-matched controls were enrolled in this study. We determined the genotypes of 17 single nucleotide polymorphisms (SNPs) from 11 genes of caspase family by the improved multiplex ligation detection reaction (iMLDR) method. A model-based single SNP frequentist test and haplotype association studies were performed to evaluate the association between SNPs and PsV.

Results. In the single SNP tests, rs6704688 in CASP8 was significantly associated with psoriasis vulgaris (PsV) in Han population of northeastern China (P = 0.0169, P' = 0.0179 under the additive model; P = 0.0126, P' = 0.0149 under the heterozygous model). In haplotype analyses, the CASP7 haplotype GC was found to be associated with PsV risk (case group versus control group, 47.2% versus 54.4%, respectively, p = 0.0149). Conclusions. Our study presented that the gene polymorphisms of CASP7 and CASP8 were significantly associated with PsV in Han population of northeastern China, which implied the functional relationship between PsV and caspase genes. CASP8 and CASP7 SNPs could be new potential biomarkers for risk stratification and prevention of PsV.

1. Introduction

Psoriasis is a common chronic relapsing inflammatory skin disorder that is induced and sustained by lymphocytes infiltrating the skin, affecting about 2-3% of the population [1, 2]. Although its etiology is not clear, we know that it is not only a skin-restricted disease but also a systemic inflammatory immune disorder which is influenced by various genetic and environmental factors [3]. As with many autoimmune diseases, abnormal apoptosis has important implications in psoriasis biology [4, 5]. The data show that, in addition to the hyperproliferation of keratinocytes, deceleration in keratinocyte apoptosis is also a significant pathological change observed in psoriasis [6–8]. Keratinocytes from the lesion of psoriasis are resistant to induction of apoptosis compared to keratinocytes from normal skin [9]. However, the current data on the apoptosis of psoriatic keratinocytes is limited. Therefore, there is a need to better understand the mechanisms of apoptosis in the progression of psoriasis and to identify potential biomarkers for prognostic prediction.

Apoptosis, a unique physiological process genetically controlling programmed cell death, is essential for maintaining normal tissue homeostasis, cell differentiation, and development [4]. In the skin, the death of apoptotic cells regulates keratinocyte proliferation and the formation of the stratum corneum [10]. Caspases (CASP) are cysteine-dependent aspartate-specific proteases that play a central role in the induction, transduction, and amplification of apoptotic signals in cells and thus determination of cell fate [11, 12]. CASPs can be generally classified into three groups based on their biological functions: inflammatory CASPs (CASP1, CASP4, CASP15, CASP11, and CASP12), apoptosis initiator (CASP2, CASP8, CASP9, and CASP10), and apoptosis executioner (CASP3, CASP6, and CASP7). CASP14 plays an
important role in the terminal differentiation of epidermal keratinocytes [13]. The apoptotic pathways involved in keratinocyte apoptosis include several mechanisms. But all have to go through the caspase family. CASPs are sequentially activated in apoptosis, and any CASP may lead to apoptosis aberration. The increase of epidermal thickness in psoriasis may be related to the abnormality of the apoptotic pathway [2]. Some of them have even been reported to have abnormal expression in the lesions of psoriasis [1, 14, 15].

Psoriasis is a widespread autoimmune disease and systemic inflammatory disorder with which many of comorbidities (e.g., diabetes, cardiovascular diseases, Crohn's disease, lymphoma, and cancer) are associated. Patients with psoriasis tend to have an increased risk of cancer [16]. Single nucleotide polymorphisms (SNPs) influence the expression or the activities of caspase enzymes. Several CASP genes polymorphisms have been found to be associated with kinds of cancers [17–21] and autoimmune inflammatory diseases, such as rheumatoid arthritis [5], coronary artery disease [22], Kawasaki disease [23], acute pancreatitis [24], and ischemic stroke [25], but the relationship with psoriasis has not been studied. And gene expression profiles showed significant differences in the expression of CASP7 and CASP8 between psoriatic lesions and psoriatic nonlesions. With this in mind, we designed a case-control trial to explore the relationship between CASP7 and CASP8 expression in the lesions of psoriasis [1, 14, 15].

2. Materials and Methods

2.1. Study Population. The present case-control study included 540 patients with PsV randomly enrolled from the Second Affiliated Hospital of Harbin Medical University from January 2013 to April 2017 and 612 healthy controls randomly recruited from the same hospital during routine health examinations performed during the same period. All samples we collected were between the ages of 5 and 85. The patients with PsV were diagnosed by at least two clinical dermatologists in the Department of Dermatology. All subjects were of Han Chinese descent in northeastern China; all patients underwent a standardized clinical evaluation and the healthy controls were without any known history of PsV (including three levels of relatives).

The basic information of all subjects including sex, age, and demographic characteristics (in the case group), including age of onset, severity grade, and family history (including three levels of relatives), was obtained from self-reported questionnaires and medical records. Subjects with systemic, infectious, autoimmune, atopic, or malignant disease were excluded.

All of the participants provided written informed consent before participating in the study. All the procedures followed were in accordance with the ethical standards of the 2nd Affiliated Hospital of Harbin Medical University on human experimentation. The Ethics Committees of the 2nd Affiliated Hospital of Harbin Medical University approved this study. All procedures were in accordance with the 1975 Declaration of Helsinki and its later amendments.

2.2. DNA Extraction. Genomic DNA was prepared from 2 mL of intravenous whole blood using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA samples were stored in a freezer (Mitsubishi, Japan) at −20°C.

2.3. Selection of Polymorphisms. Seventeen polymorphisms in 11 CASP genes were examined. We did not find any SNPs in CASP2 with the appropriate allele frequency in Han population of northeastern China. CASP11, CASP13, and CASP15 do not exist in humans. For the 17 common SNPs, 10 polymorphisms (CASP3 rs2705897 and rs4647610, CASP5 rs507879, CASP7 rs2227310, CASP8 rs6704688 and rs2293554, CASP9 rs4233532 and rs1052576, and CASP10 rs12613347 and rs13006529) were selected based on the literature suggesting that these SNPs were associated with the risk of various cancers and autoimmune diseases. For CASP1, CASP4, CASP6, CASP7, CASP12, and CASP14, we utilized the International HapMap Project database (http://hapmap.ncbi.nlm.nih.gov/) and the NCBI dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/) to select potentially functional SNPs. We set the cutoff at a minor allele frequency of more than 0.05 for the Han Chinese in Beijing (HCB) population and linkage disequilibrium (LD) patterns with r² values of more than 0.8. Based on the different distributions of SNPs, seven representative common SNPs (CASP1 rs2282659, CASP4 rs547584 and rs672016, CASP6 rs5030545, CASP7 rs17090911, CASP12 rs506601, and CASP14 rs3181304) were finally selected for genotyping.

2.4. Genotyping. Genotyping was performed by the Improved Multiple Ligase Detection Reaction technique developed by Genesky Biotechnologies Inc. (Shanghai, China) [26]. Target DNA sequences were amplified using a multiplex polymerase chain reaction (PCR) method with specific primers and probes. The details are shown in Supplementary Tables S1 and S2.

Data analysis was carried out using GeneMapper Software version 4.1. For quality control, genotyping was carried out without knowledge of group status. We randomly selected about 10% of samples for further verification and obtained a concordance rate of 100%.

2.5. Statistics. The demographic characteristics were calculated by SPSS version 19.0 (IBM, Chicago, IL, USA). χ² tests and Student’s t-tests were used to compare clinical characteristics between the two groups. The Hardy-Weinberg equilibrium (HWE; evaluated at < 0.001) test was determined using χ² tests. Associations between candidate SNPs and PsV risk were determined based on the distributions of allele and genotypic frequencies along with the additive, dominant, recessive, and heterozygous models. Multiple logistic regressions analyses were used to analyze the genotype frequency between groups. The gene interaction analysis (gene epistasis analysis) was performed using the Epistasis Module of Plink. Odds ratios and 95% confidence intervals were calculated to determine the relative risk of PsV, and 10,000 permutations were performed for the multiple test correction. The LD and haplotype were computed and constructed from the
### 3. Results

#### 3.1. Participants

Basic information and the clinical characteristics of the participants are shown in Table 1. No significant differences were observed in these factors between the two groups.

#### 3.2. Single SNP Analysis

The genotype and allele frequencies of the SNPs in the PsV group and control group are shown in Tables 2 and 3. All genotype distributions of cases and controls passed the H-W test. For the CASP8 rs6704688 polymorphism, the major C allele (risk allele) was present in 76.9% of patients and 75.3% of controls. The additive model (C versus T) distribution and the heterozygous model (TT + CC versus TC) distribution were significantly different between cases and controls ($P = 0.0169$, $P' = 0.0179$; $P = 0.0126$, and $P' = 0.0149$, respectively). No associations between the other 16 SNPs and the risk of PsV were observed in this study. However, there were correlations among CASP8 rs6704688 in genotypes of these polymorphic markers by Haploview 4.0, and LD $r^2$ values were limited to 0.8. All comparisons were two sided, and results with $P$ values of less than 0.05 were considered statistically significant.
Table 3: The single SNP association studies result of CASPs in cases and controls.

| Gene Symbol | rs2282659 | rs2705897 | rs4647610 | rs547844 | rs672016 | rs507879 | rs5030545 | rs1709991 |
|-------------|-----------|-----------|-----------|----------|----------|----------|-----------|----------|
| CASPI       |           |           |           |          |          |          |           |          |
| Genotype    | GG        | GA        | AA        | G/A      | TT       | TC       | CC        | T/C      |
| H-W P Value | 0.7871    | 0.8629    | 0.8629    | 0.1773   | 0.886    | 0.886    | 1         | 0.822    |
| P Value     | 0.5945    | 0.1953    | 0.3451    | 0.8180   | 0.8317   | 0.3841   | 0.8180    | 0.804    |
| Adjusted P  | 0.6279    | 0.2205    | 0.3884    | 0.2857   | 0.3843   | 0.4362   | 0.4632    | 0.804    |
| Statistical Model | Additive | Additive | Additive | Additive | Additive | Additive | Additive | Additive |
| p Value     | 0.8650    | 0.3765    | 0.5413    | 0.5322   | 0.5789   | 0.4794   | 0.6168    | 0.4295   |
| OR (95%CI)  | 0.8813    | 0.3845    | 0.5451    | 0.5412   | 0.5968   | 0.5470   | 0.6265    | 0.6065   |
| OR (95%CI)  |          |          |          |          |          |          |          |          |
| Statistical Model | Additive | Additive | Additive | Additive | Additive | Additive | Additive | Additive |
| p Value     | 0.8528    | 0.1609    | 0.1632    | 0.1953   | 0.8180   | 0.3439   | 0.3845    | 0.4295   |
| OR (95%CI)  | 1.083     | 0.1908    | 1.221     | 1.083    | 0.9057   | 0.3586   | 0.8991    | 1.043    |
| OR (95%CI)  |          |          |          |          |          |          |          |          |
| Statistical Model | Additive | Additive | Additive | Additive | Additive | Additive | Additive | Additive |
| p Value     | 0.6057    | 0.1953    | 0.1832    | 0.2056   | 0.8057   | 0.3586   | 0.7998    |          |
| OR (95%CI)  | (0.812,1.444) | (0.899,1.463) | (0.899,1.463) |          | (0.902,1.653) | (0.899,1.463) |          |          |
| OR (95%CI)  |          |          |          |          |          |          |          |          |
| Statistical Model | Additive | Additive | Additive | Additive | Additive | Additive | Additive | Additive |
| p Value     | 0.5922    | 0.7222    | 0.1832    | 0.2056   | 0.8057   | 0.3586   | 0.7998    |          |
| OR (95%CI)  |          |          |          |          |          |          |          |          |
| Statistical Model | Additive | Additive | Additive | Additive | Additive | Additive | Additive | Additive |
| p Value     | 0.5968    | 0.5968    | 0.5968    | 0.5968   | 0.5968   | 0.5968   | 0.5968    | 0.5968   |
| OR (95%CI)  |          |          |          |          |          |          |          |          |
| Statistical Model | Additive | Additive | Additive | Additive | Additive | Additive | Additive | Additive |
| p Value     | 0.5412    | 0.5412    | 0.5412    | 0.5412   | 0.5412   | 0.5412   | 0.5412    | 0.5412   |
| OR (95%CI)  |          |          |          |          |          |          |          |          |
Table 3: Continued.

| Gene Symbol | Genotype | Cases (n=540) | Controls (n=612) | H-W P Value | P Value | Adjusted P Value | Statistical Model | OR (95%CI) |
|-------------|----------|---------------|------------------|-------------|---------|-----------------|------------------|------------|
| rs2227310   |          |               |                  |             |         |                 |                  |            |
| GG          |          | 104(0.193)    | 88(0.144)        | 0.5244      | 0.0894  | 0.0919          | Additive         | 0.2164     |
| GC          |          | 260(0.481)    | 294(0.480)       |             |         |                 |                  | 0.2147     |
| CC          |          | 176(0.326)    | 230(0.376)       |             |         |                 |                  |            |
| G/C         |          | 468/612       | 470/754          |             |         |                 |                  |            |
|             |          | 0.433/0.567   | 0.384/0.616      |             |         |                 |                  |            |
| rs6704688   |          |               |                  |             |         |                 |                  |            |
| TT          |          | 40(0.074)     | 24(0.039)        |             |         |                 | Additive         | 0.0169*    |
| TC          |          | 170(0.315)    | 254(0.415)       | 0.8725      | 0.5429  | 0.5777          | Dominant         | 0.0679     |
| CC          |          | 330(0.611)    | 334(0.546)       |             |         |                 |                  | 0.0894     |
| T/C         |          | 250/830       | 302/922          |             |         |                 |                  | 0.0919     |
|             |          | 0.231/0.769   | 0.247/0.753      |             |         |                 |                  |            |
| rs2293554   |          |               |                  |             |         |                 |                  |            |
| TT          |          | 332(0.615)    | 358(0.585)       | 0.546       | 0.3999  | 0.4151          | Additive         | 0.6952     |
| TG          |          | 178(0.329)    | 212(0.346)       |             |         |                 |                  | 0.6784     |
| GG          |          | 30(0.056)     | 42(0.069)        |             |         |                 |                  |            |
| T/G         |          | 842/238       | 928/296          |             |         |                 |                  |            |
|             |          | 0.780/0.220   | 0.758/0.242      |             |         |                 |                  |            |
| rs4233532   |          |               |                  |             |         |                 |                  |            |
| TT          |          | 176(0.326)    | 224(0.366)       |             |         |                 | Additive         | 0.2005     |
| TC          |          | 278(0.515)    | 270(0.441)       | 0.0447      | 0.9114  | 0.9544          | Dominant         | 0.3129     |
| CC          |          | 86(0.159)     | 118(0.193)       |             |         |                 |                  | 0.3380     |
| T/C         |          | 630/450       | 718/506          |             |         |                 |                  | 1.013      |
|             |          | 0.584/0.416   | 0.587/0.413      |             |         |                 |                  |            |
| rs1052576   |          |               |                  |             |         |                 |                  |            |
| TT          |          | 70(0.130)     | 98(0.160)        |             |         |                 | Additive         | 0.2790     |
| TC          |          | 258(0.478)    | 254(0.415)       | 1           | 0.9761  | 0.9999          | Dominant         | 0.2994     |
| CC          |          | 212(0.392)    | 260(0.425)       |             |         |                 |                  | 0.3467     |
| T/C         |          | 398/682       | 450/774          |             |         |                 |                  | 0.996      |
|             |          | 0.369/0.631   | 0.368/0.632      |             |         |                 |                  |            |
| rs12613347  |          |               |                  |             |         |                 |                  |            |
| TT          |          | 74(0.137)     | 82(0.134)        |             |         |                 | Additive         | 0.9752     |
| TC          |          | 238(0.441)    | 266(0.435)       | 0.6406      | 0.8325  | 0.8550          | Dominant         | 0.9150     |
| CC          |          | 228(0.422)    | 264(0.431)       |             |         |                 |                  | 0.9998     |
| T/C         |          | 386/694       | 430/794          |             |         |                 |                  | 0.974      |
|             |          | 0.357/0.643   | 0.351/0.649      |             |         |                 |                  |            |
| rs13006529  |          |               |                  |             |         |                 |                  |            |
| TT          |          | 342(0.633)    | 366(0.598)       |             |         |                 | Additive         | 0.8046     |
| TA          |          | 164(0.304)    | 226(0.369)       | 0.4431      | 0.9179  | 0.9452          | Dominant         | 0.3849     |
| AA          |          | 34(0.063)     | 20(0.033)        |             |         |                 |                  | 0.3863     |
| T/A         |          | 848/232       | 958/266          |             |         |                 |                  | 0.985      |
|             |          | 0.785/0.215   | 0.783/0.217      |             |         |                 |                  |            |
| rs13006601  |          |               |                  |             |         |                 |                  |            |
| TT          |          | 48(0.089)     | 48(0.078)        |             |         |                 | Additive         | 0.5086     |
| TA          |          | 230(0.426)    | 290(0.484)       | 0.6716      | 0.6101  | 0.6427          | Dominant         | 0.6507     |
| AA          |          | 262(0.485)    | 274(0.448)       |             |         |                 |                  | 0.7603     |
| T/A         |          | 326/754       | 386/838          |             |         |                 |                  | 0.8745     |
|             |          | 0.302/0.698   | 0.315/0.685      |             |         |                 |                  |            |
Table 3: Continued.

| Gene Symbol | Genotype | Cases (n=540) | Controls (n=612) | H-W P Value | P Value | Adjusted P Value | Statistical Model | P | P’ | OR (95%CI) |
|-------------|----------|---------------|-----------------|-------------|---------|-----------------|------------------|---|----|-----------|
| CASP14      | rs3181304 |               |                 |             |         |                 |                  |   |    |           |
|             | GG       | 120 (0.222)   | 104 (0.170)     | Additive    | 0.2007  | 0.2129          |                   |   |    |           |
|             | GA       | 230 (0.426)   | 298 (0.487)     | Dominant    | 0.1139  | 0.1425          |                   |   |    |           |
|             | AA       | 190 (0.352)   | 210 (0.343)     | Recessive   | 0.8265  | 0.8582          | (0.724, 1.156)    |   |    |           |
|             | G/A      | 470/610       | 506/718         | heterozygous| 0.1424  | 0.1547          |                   |   |    |           |

*: Significant, compared by paired t test; H-W: Hardy–Weinberg equilibrium test; P: model-based statistical p value; P’: p value adjusted by permutation; OR: odds ratio; and 95% CI: 95% confidence interval.

Table 4: Significant results of intergenic interaction analysis of all samples.

| SNP (Chr.) | SNP (Chr.) | OR_INT | Chi-square | P    |
|------------|------------|--------|------------|------|
| rs1261347(2) | rs672016(II) | 1.88   | 6.8995     | 0.0086 |
| rs13006529(2) | rs506601(II) | 0.384  | 6.6773     | 0.0098 |
| rs13006529(2) | rs4647610(4) | 3.039  | 6.0643     | 0.0138 |
| rs4233532(1) | rs13006529(2) | 0.430  | 4.9705     | 0.0258 |
| rs6704688(2) | rs4647610(4) | 0.54   | 4.6476     | 0.0311 |
| rs1261347(2) | rs2282659(11) | 0.41   | 4.6218     | 0.0316 |
| rs5066001(II) | rs2282659(11) | 0.43   | 4.5832     | 0.0323 |
| rs6704688(2) | rs672016(II) | 0.58   | 4.5381     | 0.0332 |

SNP: single nucleotide polymorphism; Chr.: chromosome of single nucleotide polymorphism; OR_INT: odds ratio for interaction; P: p-value.

Table 5: Significant results of intergenic interaction analysis of samples less than 40 years old.

| SNP (Chr.) | SNP (Chr.) | OR_INT | Chi-square | P    |
|------------|------------|--------|------------|------|
| rs13006529(2) | rs6704688(2) | 4.85   | 8.9000     | 0.0029 |
| rs5030545(4) | rs2705897(4) | 0.12   | 6.1767     | 0.0129 |
| rs4647610(4) | rs547858(II) | 2.90   | 5.9144     | 0.0150 |
| rs2293554(2) | rs4647610(4) | 2.45   | 5.2208     | 0.0223 |
| rs4233532(1) | rs1052576(II) | 0.50   | 4.9522     | 0.0261 |
| rs5030545(4) | rs4647610(4) | 0.24   | 4.6407     | 0.0319 |
| rs6704688(2) | rs2282659(II) | 0.34   | 4.2747     | 0.0387 |
| rs5030545(4) | rs1709091I(II) | 0.08  | 4.0495     | 0.0442 |
| rs4233532(1) | rs2227310(II) | 1.82  | 3.9662     | 0.0464 |
| rs13006529(2) | rs2282659(II) | 0.34  | 3.9280     | 0.0475 |

SNP: single nucleotide polymorphism; Chr.: chromosome of single nucleotide polymorphism; OR_INT: odds ratio for interaction; P: p-value.

3.3. Interaction Analysis. We performed a gene–gene interaction analysis on all samples. We found that the interaction of CASP10 - CASPI, CASP10 - CASP3, CASP10 - CASP4, CASP10 - CASP9, CASP10 - CASP12, CASP8 - CASP3, CASP8 - CASP4, and CASP1 - CASP12 is important for the risk of psoriasis (Table 4). Additional results that were not found to be significant were shown in Supplementary Materials (Table S3). The age group under 40 is a high-risk age group for psoriasis. The analysis of intergene interactions in this age group found that the interaction between 10 pairs of genes was significant for the risk of psoriasis. They are the interaction of CASP8 - CASP10, CASP3 - CASP4, CASP3 - CASP6, CASP3-CASP8, CASPI - CASP8, CASPI - CASP10, CASP6 - CASP7, and CASP7 - CASP9 (Table 5). Additional results that were not found to be significant were shown in Supplementary Materials (Table S4).

3.4. Haplotype Analysis. The haplotype blocks were imputed by Haplovieview for each of the 11 genes and we obtained four haplotypes in CASP3 and CASP4 and three haplotypes in
Table 6: The haplotype analyses result of CASPs in cases and controls.

| Gene | Haplotype | Freq. | Case, Control Ratio | Counts Case, Control | Case, Control Frequencies | Chi Square | P Value |
|------|-----------|-------|---------------------|----------------------|--------------------------|------------|---------|
| CASP3 | TT | 0.646 | 341.8 : 198.2, 401.9 : 210.1 | 0.633, 0.657 | 0.712 | 0.3989 |
| CASP3 | GC | 0.166 | 99.8 : 440.2, 90.9 : 521.1 | 0.174, 0.180 | 0.057 | 0.818 |
| CASP3 | GC | 0.177 | 94.2 : 445.8, 110.1 : 501.9 | 0.185, 0.194 | 2.721 | 0.099 |
| CASP3 | GT | 0.012 | 4.2 : 535.8, 91.1 : 602.9 | 0.008, 0.015 | 1.234 | 0.2666 |
| CASP4 | TC | 0.633 | 334.5 : 205.5, 393.5 : 218.5 | 0.619, 0.643 | 0.685 | 0.408 |
| CASP4 | TG | 0.201 | 109.5 : 430.5, 122.5 : 489.5 | 0.203, 0.200 | 0.013 | 0.911 |
| CASP4 | CC | 0.143 | 82.5 : 457.5, 82.5 : 529.5 | 0.153, 0.135 | 0.758 | 0.384 |
| CASP4 | CG | 0.023 | 13.5 : 526.5, 13.5 : 598.5 | 0.025, 0.022 | 0.106 | 0.7446 |
| CASP7 | GC | 0.51 | 255.0 : 285.0, 333.0 : 279.0 | 0.472, 0.544 | 5.934 | 0.0149 |
| CASP7 | GG | 0.407 | 234.0 : 306.0, 235.0 : 377.0 | 0.433, 0.384 | 2.894 | 0.0889 |
| CASP7 | AC | 0.082 | 51.0 : 489.0, 44.0 : 568.0 | 0.094, 0.072 | 1.928 | 0.165 |
| CASP8 | CT | 0.546 | 304.0 : 236.0, 325.2 : 286.8 | 0.563, 0.531 | 1.157 | 0.282 |
| CASP8 | TT | 0.222 | 117.0 : 423.0, 138.8 : 473.2 | 0.217, 0.227 | 0.172 | 0.6787 |
| CASP8 | CG | 0.214 | 111.0 : 429.0, 135.8 : 476.2 | 0.205, 0.222 | 0.457 | 0.4991 |
| CASP9 | TC | 0.582 | 314.9 : 225.1, 355.9 : 256.1 | 0.583, 0.582 | 0.003 | 0.9559 |
| CASP9 | CT | 0.365 | 198.9 : 341.1, 221.9 : 390.1 | 0.368, 0.363 | 0.041 | 0.8397 |
| CASP9 | CC | 0.05 | 26.1 : 513.9, 31.1 : 580.9 | 0.048, 0.051 | 0.037 | 0.8465 |
| CASP10 | CT | 0.431 | 232.3 : 307.7, 264.4 : 374.6 | 0.430, 0.432 | 0.004 | 0.9506 |
| CASP10 | TT | 0.353 | 191.7 : 348.3, 214.6 : 397.4 | 0.355, 0.351 | 0.023 | 0.8783 |
| CASP10 | CA | 0.215 | 114.7 : 425.3, 132.6 : 479.4 | 0.212, 0.217 | 0.031 | 0.8596 |

*: significant; Freq.: frequencies.

CASP7, CASP8, CASP9, and CASP10. The results are shown in Table 6. The results showed that, among the 21 haplotypes, the CASP3 haplotype TT and CASP4 haplotype TC were the two most common in the case and control groups (TT frequencies of 0.633 and 0.657, respectively, and TC frequencies of 0.619 and 0.643, respectively). The CASP7 haplotype GC was significantly different in patients with PsV than in controls (47.2% versus 54.4%, \( p = 0.0149 \)). We did not identify the other haplotypes to be significantly associated with PsV (\( P > 0.05 \)).

We also used Haploview to calculate the haplotypes of individual genes on the same chromosome, estimated the haplotype frequencies, and compared the haplotype distribution between the patients and control groups. We found that the haplotype frequency of CASP3 (rs2705897) on chromosome 4, CASP7 (rs17090911) on chromosome 10, CASP10 (rs12613347) on chromosome 2, and CASP12 (rs506601) on chromosome 11 was significantly different between the patients group and the control group. The results are shown in Table 7. We performed the same analysis for the age group under 40 years old and found that the haplotype frequency of CASP7 (rs17090911) on chromosome 10, CASP9 (rs4233532) on chromosome 1, CASP10 (rs12613347) on chromosome 2, and CASP12 (rs506601) on chromosome 11 was significantly different between the patients group and the control group. The results are shown in Table 8.

4. Discussion

Genome-wide association studies (GWASs) have identified numerous single nucleotide polymorphisms (SNPs) associated with psoriasis risk [27, 28]; however, our study was the first to evaluate the association of CASP family genes SNPs with risk of PsV in Han population in northeast China. We found that CASP8 rs6704688 and the CASP7 haplotype GC were significantly different between cases and controls. These findings suggested that polymorphisms in CASP7 and CASP8 were associated with PsV risk in Han population of northeastern China and provide new insights into the importance of CASP7 and CASP8 in psoriasis susceptibility.

The apoptotic pathways of keratinocytes mainly include two mechanisms, and the process is regulated by a variety of proteins, genes, and internal and external stimuli. The “extrinsic” pathway is triggered by the binding of Fas ligand (FasL) or tumor necrosis factor (TNF) to membrane death receptors and these membrane death receptors recruit adapter molecules resulting in the activation of CASP8 [10]. The activated CASP8 then initiates a downstream apoptotic cascade by cleaving CASP3 and/or CASP7 leading to apoptosis. The “intrinsic” pathway includes mitochondrial release of cytochrome c and, along with the cofactor Apaf-1, the formation of an activated CASP9 apoptosome [10]. CASP3 and/or CASP7 are then activated, ultimately leading to apoptosis. Finally, both pathways end with DNA cleavage,
| Chr. | SNP      | Haplotype | Ca-freq. | N-Ca (540) | Co-freq. | N-Co (612) | P     |
|------|----------|-----------|----------|------------|----------|------------|-------|
| 1    | rs4233532| CC        | 0.048    | 23.92      | 0.053    | 32.436     | 0.9226|
|      | rs1052576| CT        | 0.369    | 199.26     | 0.364    | 222.768    |       |
|      |          | TC        | 0.583    | 314.82     | 0.583    | 356.796    |       |
| 11   | rs506601 | ACCCG     | 0.034    | 18.36      | 0.027    | 16.524     | 0.0093|
|      | rs547584 | ACCTA     | 0.116    | 62.64      | 0.102    | 62.424     |       |
|      | rs672016 | AGTAA     | 0.028    | 15.12      | 0.023    | 14.076     |       |
|      | rs507879 | ATCCCG    | 0.057    | 30.78      | 0.067    | 41.004     |       |
|      | rs2282659| ATCTA     | 0.265    | 143.1      | 0.277    | 169.524    |       |
|      |          | TTCCCG    | 0.033    | 17.82      | 0.047    | 28.764     |       |
|      |          | TTCTA     | 0.245    | 132.3      | 0.238    | 145.656    |       |
|      |          | TTCTG     | 0.018    | 9.72       | 0        | 0          |       |
|      |          | TTTGA     | 0        | 0          | 0.017    | 10.404     |       |
| 10   | rs1709091| AC        | 0.094    | 50.76      | 0.072    | 44.064     | 0.0448|
|      | rs2227310| GC        | 0.473    | 255.42     | 0.544    | 332.928    |       |
|      |          | GG        | 0.433    | 233.82     | 0.384    | 235.008    |       |
| 2    | rs12613347| CACT      | 0        | 0          | 0.012    | 7.344      | 0.0038|
|      | rs13006529| CATG      | 0        | 0          | 0.01     | 6.12       |       |
|      | rs6704688 | CATT      | 0.2      | 108        | 0.196    | 119.952    |       |
|      | rs2293554 | CTCG      | 0.159    | 83.86      | 0.164    | 100.368    |       |
|      |          | CTCT      | 0.263    | 142.02     | 0.248    | 151.776    |       |
|      |          | CTGT      | 0.023    | 12.42      | 0.023    | 14.076     |       |
|      |          | TTCG      | 0.05     | 27         | 0.064    | 39.168     |       |
|      |          | TTCT      | 0.305    | 164.7      | 0.268    | 164.016    |       |
|      |          | TTCTT     | 0        | 0          | 0.015    | 9.18       |       |
| 4    | rs2705897 | CGC       | 0.179    | 96.66      | 0.124    | 75.888     | 0.00004|
Table 8: Haplotype analysis of SNPs on the same chromosome for samples under 40 years old.

| Chr | SNP       | Haplotype | Ca-freq | N-Ca (439) | Co-freq | N-Co (216) | P     |
|-----|-----------|-----------|---------|------------|---------|------------|-------|
| 1   | rs4233532 | CC        | 0.545   | 239.255    | 0.051   | 11.016     | 0.00000 |
|     | rs1052576 | CT        | 0.401   | 176.039    | 0.338   | 73.008     |       |
|     |           | TC        | 0.054   | 23.706     | 0.611   | 131.976    |       |
| 11  | rs506601  | ACCCG     | 0.042   | 18.438     | 0.019   | 4.104      | 0.00098 |
|     | rs547584  | ACCTA     | 0.119   | 52.241     | 0.105   | 22.68      |       |
|     | rs672016  | AGTA      | 0.035   | 15.365     | 0.024   | 5.184      |       |
|     |           | ATCCA     | 0       | 0          | 0.019   | 4.104      |       |
|     | rs507879  | ATCCG     | 0.027   | 11.853     | 0.058   | 12.528     |       |
|     | rs2282659 | ATCTA     | 0.309   | 135.651    | 0.224   | 48.384     |       |
|     |           | ATCTG     | 0.015   | 6.585      | 0.038   | 8.208      |       |
|     |           | ATGCG     | 0.053   | 23.267     | 0.043   | 9.288      |       |
|     |           | ATGTA     | 0.117   | 51.363     | 0.172   | 37.152     |       |
|     |           | TTCCG     | 0.026   | 11.414     | 0.054   | 11.664     |       |
|     |           | TTCTA     | 0.227   | 99.653     | 0.229   | 49.464     |       |
|     |           | TTCTG     | 0.018   | 7.902      | 0       | 0          |       |
|     |           | TTGTA     | 0.002   | 5.268      | 0.015   | 3.24       |       |
| 10  | rs17090911| AC        | 0.094   | 41.266     | 0.102   | 22.032     | 0.08798 |
|     | rs2227310 | GC        | 0.436   | 191.404    | 0.518   | 111.888    |       |
|     |           | GG        | 0.47    | 206.33     | 0.38    | 82.08      |       |
| 2   | rs12613347| CACT      | 0       | 0          | 0.015   | 3.24       | 0.00000 |
|     | rs13006529| CATT      | 0.176   | 77.264     | 0.187   | 40.392     |       |
|     | rs6704688 | CTCC      | 0       | 0          | 0.161   | 34.776     |       |
|     | rs2293554 | CTGC      | 0.198   | 86.922     | 0       | 0          |       |
|     |           | CTCT      | 0.289   | 126.871    | 0.294   | 63.504     |       |
|     |           | CTGT      | 0       | 0          | 0.028   | 6.048      |       |
|     |           | TTCC      | 0       | 0          | 0.073   | 15.768     |       |
|     |           | TTCT      | 0.075   | 32.925     | 0       | 0          |       |
|     |           | TTGG      | 0.262   | 115.018    | 0.226   | 48.816     |       |
|     |           | TTGG      | 0.016   | 6.048      | 0       | 0          |       |
| 4   | rs2705897 | CGC       | 0.176   | 77.264     | 0.107   | 23.112     | 0.00001 |
|     | rs4647610 | CGT       | 0       | 0          | 0.011   | 2.376      |       |
|     | rs5030545 | CTC       | 0.183   | 80.337     | 0.182   | 39.312     |       |
|     |           | CCT       | 0.533   | 233.987    | 0.644   | 139.104    |       |
|     |           | TGC       | 0       | 0          | 0.02    | 4.32       |       |
|     |           | TTC       | 0.014   | 6.146      | 0.016   | 3.456      |       |
|     |           | TTT       | 0.094   | 41.266     | 0.02    | 4.32       |       |

Chr, Chromosome; SNP, single nucleotide polymorphism; N-Ca, number of case group; Ca-freq, case group frequencies; N-Co, Number of control group; Co-freq, control group frequencies; P, p value.

maintain the inflammatory process of psoriasis [33]. Studies have shown the DEL allele and DEL carrier of rs3834129 had negative associations with cancer susceptibility in Asian populations and rs6704688 is associated with reduced cancer risk [13]. Combined with our study, we predicted that the T allele and T-carriers of rs6704688 were negatively correlated with the susceptibility to PsV from the additive model, whereas the heterozygous TC genotype had a protective effect from the heterozygous model. In the follow-up, we will conduct a detailed study of rs3834129.

Given the role of CASP7 in apoptosis and inflammation, the associations among CASP7 and autoimmune diseases and cancer susceptibility are important. Their relevance has been reported in insulin-dependent diabetes mellitus, rheumatoid arthritis, childhood leukemia, and different types of human cancers [18, 21]. Soung et al. found that a CASP7 gene inactivating mutation (70 Cys to Tyr mutant) could lead to loss of apoptotic function [18]. Thus, we hypothesized that genetic variations in CASP7 may act as strong apoptosis signals to block or delay the apoptosis of keratinocyte cells in PsV. The frequency of the haplotype GC in cases was lower than controls, which implied a protective role with regard to cases versus controls.

This study had some limitations. First, it is a hospital-based case-control study and the sample size was relatively small, which may limit the statistical power to explore
real association. In the future, we need to further study the larger sample size and ethnicity to validate our results. Next, we tested limited polymorphisms that were representative in CASP genes; these polymorphisms may not be the critical loci for psoriasis but may instead be nearby in LD with a causative locus. Studies on the correlations of more gene loci are needed. Additionally, more comprehensive studies, such as additional clinical studies and functional analyses, are needed to explain and supplement our findings. Despite these limitations, our study demonstrated the association between CASP7 and CASP8 polymorphisms and the PsV risk.

5. Conclusions

In conclusion, our findings demonstrated that CASP7 and CASP8 gene polymorphisms were associated with PsV risk in Han population of northeastern China. This is the first study describing the relationships between genetic polymorphisms in CASP family genes and PsV susceptibility in Han population in northeast China. CASP7 and CASP8 gene polymorphisms may be involved in the etiopathogenesis of PsV, and analysis of these polymorphisms could provide new insights into the diagnosis and treatment of PsV.

Data Availability

According to the informed consent signed by the volunteers, the aggregated SNP genotype frequency and allele frequency data in the manuscript can be available freely by the academic community. The SNP genotype and the clinical information can be claimed by e-mail after security audit.

Conflicts of Interest

There are no conflicts of interest.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (no. 81773320). We are most grateful to the members of all the families who have so willingly participated in this study.

Supplementary Materials

The supplementary materials contain several experimental data and experimental results that are closely related to the content of the manuscript. They are relatively unimportant or large or negative results, but they are still very meaningful for manuscripts. (Supplementary Materials)

References

[1] S. M. Bebars, D. R. Al-Sharaky, M. A. Gaber, and D. R. Afify, “Immunohistochemical expression of caspase 3 in psoriasis,” Journal of Clinical and Diagnostic Research, vol. 11, Article ID EC01-5, 2017.
[2] M. El-Domyati, N. H. Moftah, G. A. Nasif, H. M. Abdel-Wahab, M. T. Barakat, and R. T. Abdel-Aziz, “Evaluation of apoptosis regulatory proteins in response to PUVA therapy for psoriasis,” Photodermatology, Photoimmunology & Photomedicine, vol. 29, no. 1, pp. 18–26, 2013.
[3] N. A. A. de Fraga, M. F. P. de Oliveira, I. Follador, B. O. de Rocha, and V. R. Rêgo, "Psoriasis and uveitis: a literature review," Anais Brasileiros de Dermatologia, vol. 87, no. 6, pp. 877–883, 2012.
[4] J. D. Kuhlmann, A. Bankfalvi, K. W. Schmid et al., “Prognostic relevance of caspase 8–652 6N InsDel and Asp302His polymorphisms for breast cancer,” BMC Cancer, vol. 16, no. 1, p. 618, 2016.
[5] J. R. García-Lozano, B. Torres, O. Fernández et al., “Caspase 7 influences susceptibility to rheumatoid arthritis,” Rheumatology, vol. 46, no. 8, pp. 1243–1247, 2007.
[6] X. Zeng, J. ZhaO, X. Wu et al., “PageRank analysis reveals topologically expressed genes correspond to psoriasis and their functions are associated with apoptosis resistance,” Molecular Medicine Reports, vol. 13, no. 5, pp. 3969–3976, 2016.
[7] T. Elango, A. Thirupathi, S. Subramanian, P. Ethira, H. Dayalan, and P. Gnanaraj, “Methotrexate treatment provokes apoptosis of proliferating keratinocyte in psoriasis patients,” Clinical and Experimental Medicine, vol. 17, no. 3, pp. 371–381, 2017.
[8] Y. Deng, C. Chang, and Q. Lu, “The inflammatory response in psoriasis: a comprehensive review,” Clinical Reviews in Allergy & Immunology, vol. 50, no. 3, pp. 377–389, 2016.
[9] L. Borska, C. Andrys, M. Chmelarova et al., “Roles of miR-31 and endothelin-1 in psoriasis vulgaris: pathophysiological functions and potential biomarkers,” Physiological Research, 2017.
[10] M. Kastelan, L. Pripić-Massari, and Brajic L., “Apoptosis in psoriasis,” Acta Dermatovenerologica Croatica, vol. 17, no. 3, pp. 182–186, 2009.
[11] E. Kuranaga, “Beyond apoptosis: caspase regulatory mechanisms and functions in vivo,” Genes to Cells, vol. 17, no. 2, pp. 83–97, 2012.
[12] I. Chowdhury, B. Tharakan, and G. K. Bhat, “Caspases — an update,” Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology, vol. 151, no. 1, pp. 10–27, 2008.
[13] Y. J. Zhang, X. P. Zhong, Y. Chen, S. R. Liu, G. Wu, and Y. F. Liu, “Association between CASP-8 gene polymorphisms and cancer risk in some Asian population based on a HuGE review and meta-analysis,” Genetics and Molecular Research, vol. 12, no. 4, pp. 6466–6476, 2013.
[14] A. Thirupathi, T. Elango, S. Subramanian, and P. Gnanaraj, “Methotrexate regulates Th-1 response by suppressing caspase-1 and cytokines in psoriasis patients,” Clinica Chimica Acta, vol. 453, pp. 164–169, 2016.
[15] S. Lippens, M. Kockx, M. Knaapen et al., “Epidermal differentiation does not involve the pro-apoptotic executioner caspases, but is associated with caspase-14 induction and processing,” Cell Death & Differentiation, vol. 7, no. 12, pp. 1218–1224, 2000.
[16] L. Şahmatova, E. Sügis, M. Sunina et al., “Signs of innate immune activation and premature immunosenescence in psoriasis patients,” Scientific Reports, vol. 7, no. 1, p. 7553, 2017.
[17] S. Zhang, Q. Xiao, Z. Shi et al., “Caspase polymorphisms and prognosis of hepatocellular carcinoma,” PLoS ONE, vol. 12, no. 4, p. e0176802, 2017.
[18] C. Park, S. Han, K. Lee et al., “Association between CASP7 and CASP4 genetic polymorphisms and the risk of childhood leukemia,” Human Immunology, vol. 73, no. 7, pp. 736–739, 2012.
[19] J. Lin, Y. Zhang, H. Wang et al., “Genetic polymorphisms in the apoptosis-associated gene CASP3 and the risk of lung cancer in chinese population,” PLoS ONE, vol. 11, no. 10, p. e0164358, 2016.
[20] S. G. Yilmaz, F. Yencilek, A. Yildirim, E. Yencilek, and T. Isbir, “Effects of caspase 9 gene polymorphism in patients with prostate cancer,” In Vivo, vol. 31, no. 2, pp. 205–208, 2017.
[21] H. Liu, X. Jiang, M. Zhang et al., “Association of CASP9, CASP10 gene polymorphisms and tea drinking with colorectal cancer risk in the Han Chinese population,” *Journal of Zhejiang University SCIENCE B*, vol. 14, no. 1, pp. 47–57, 2013.

[22] K. K. Gundapaneni, N. Shyamala, R. K. Galimudi et al., “Poly-morphic variants of caspase genes (8 & 3) in the risk prediction of coronary artery disease,” *Gene*, vol. 627, pp. 278–283, 2017.

[23] Q. Peng, C.-H. Chen, Q. Wu, and Y. Yang, “Association of new functional SNP rs72689236 of CASP3 with kawasaki disease: a meta-Analysis,” *Zhongguo Dang Dai Er Ke Za Zhi*, vol. 15, no. 6, pp. 477–483, 2013.

[24] F. D’Oliveira Martins, B. C. Gomes, A. S. Rodrigues, and J. Rueff, “Genetic susceptibility in acute pancreatitis: genotyping of GSTM1, GSTT1, GSTP1, CASP7, CASP8, CASP9, CASP10, LTA, TNFRSF1B, and TP53 gene variants,” *Pancreas*, vol. 46, no. 1, pp. 71–76, 2017.

[25] B. Y. Lee, J. Chon, H. Kim et al., “Association between a polymorphism in CASP3 and CASP9 genes and ischemic stroke,” *Annals of Rehabilitation Medicine*, vol. 41, no. 2, p. 197, 2017.

[26] G. Thomas, R. Sinville, S. Sutton et al., “Capillary and microelectrophoretic separations of ligase detection reaction products produced from low-abundant point mutations in genomic DNA,” *Electrophoresis*, vol. 25, no. 10-11, pp. 1668–1677, 2004.

[27] X. Yin, H. Cheng, Y. Lin et al., “A weighted polygenic risk score using 14 known susceptibility variants to estimate risk and age onset of psoriasis in Han Chinese,” *PLoS ONE*, vol. 10, no. 5, Article ID e0125369, 2015.

[28] L. C. Tsoi, P. E. Stuart, C. Tian et al., “Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants,” *Nature Communications*, vol. 8, Article ID 15382, 2017.

[29] X. Zhao, C. Gu, C. Yan et al., “NALP3-inflammasome-related gene polymorphisms in patients with prehypertension and coronary atherosclerosis,” *BioMed Research International*, vol. 2016, Article ID 7395627, 10 pages, 2016.

[30] S. Y. Lee, Y. Y. Choi, J. E. Choi et al., “Polymorphisms in the caspase genes and the risk of lung cancer,” *Journal of Thoracic Oncology*, vol. 5, no. 8, pp. 1152–1158, 2010.

[31] J. Cai, Q. Ye, S. Luo et al., “CASP8 -652 6N insertion/deletion polymorphism and overall cancer risk: evidence from 49 studies,” *Oncotarget*, vol. 8, pp. 56780–56790, 2017.

[32] C. Li, H. Zhao, Z. Hu et al., “Genetic variants and haplotypes of the caspase-8 and caspase-10 genes contribute to susceptibility to cutaneous melanoma,” *Human Mutation*, vol. 29, no. 12, pp. 1443–1451, 2008.

[33] Z. Jadali and M. B. Eslami, “T cell immune responses in psori-asis,” *Iranian Journal of Allergy, Asthma and Immunology*, vol. 13, no. 4, pp. 220–230, 2014.