Validation of Susceptibility Loci for Vitiligo Identified by GWAS in the Chinese Han Population

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INTRODUCTION

Vitiligo is an organ-specific autoimmune disease directed against melanocytes. It is characterized by whitish patches on the skin (Jin et al., 2019). The disease occurs in approximately 0.5–1% of the world population (Ezzedine et al., 2012). Most patients develop vitiligo before 40 years of age (Ezzedine et al., 2015). Vitiligo is also related to many other autoimmune diseases, in particular...
autoimmune thyroid disease, type 1 diabetes, and rheumatoid arthritis (Jin et al., 2019). The etiology of vitiligo remains elusive. There are several hypotheses, but an autoimmune etiology associated with specific genetic variants is still considered the leading theory (Iannella et al., 2016). It is believed that genetic components contribute to the onset of this disease (Spritz, 2012).

In 2010, we performed a genome-wide association study (GWAS) of vitiligo in the Chinese population, identifying 6p21.33, 6q27, and 10q22.3 as susceptible loci (Quan et al., 2010). Further two-stage replication studies identified three additional susceptibility loci at 10q22.1, 11q23.3, and 12q13.2 (Tang et al., 2013). From 2010 to 2018, the Spritz group carried out several large-scale GWASs in European-derived whites and discovered 49 novel susceptibility loci that contributed to vitiligo risk (Jin et al., 2010a,b, 2012, 2016; Ben et al., 2018). However, due to the genetic heterogeneity across different ethnicities, only some of these loci have been validated in the Chinese Han population, whereas others apparently have not been (Li et al., 2015; Xu et al., 2018; Tang et al., 2019). Of the 49 reported new susceptibility loci, we selected 16 single-nucleotide polymorphisms (SNPs) from 16 loci for validation after excluding those loci validated previously (Li et al., 2015; Xu et al., 2018; Tang et al., 2019). The genotype data of the 16 SNPs in adjacent SNPs with a call rate >90% and minor allele frequency (MAF) > 0.05 in controls. For quality control, SNPs that met the following criteria were included: (1) P-value of <10^{-3}; (2) call rates >90%; (3) minor allele frequency (MAF) > 0.05 in controls; (4) P-value for Hardy–Weinberg equilibrium (P_{HWE}) > 0.01 in controls. Then we selected the reported SNPs and the most significant SNPs in the 16 loci (Table 2). Of those, the designs of the PCR primers for six SNPs failed. Finally, 26 SNPs within 16 loci were enrolled in the genotyping.

**DNA Extraction and Genotyping**

Genomic DNA was extracted from blood samples using FlexiGene DNA kits (QIAGEN, Hilden, Germany) according to the manufacturer’s protocol. After the concentration and purity were measured using a spectrophotometer, the 26 SNPs were genotyped using the Sequenom MassARRAY platform (Sequenom, San Diego, CA, United States). The procedures used for genotyping were presented in a previous study (Cai et al., 2019).

**Statistical Analysis**

PLINK 2.0 software was used to calculate the allele frequency, P-values, odds ratios (OR), and Hardy–Weinberg equilibriums (HWE) for each SNP. The association of vitiligo with the SNPs was analyzed by comparing the MAF between patients and controls. The statistical power was calculated using Power and Sample Size Calculation software using either the sample size, the MAFs observed in the Chinese controls, and the ORs previously reported for European-derived whites, or the ORs in our GWAS and the imputed results for the Chinese Han when they were not provided for the white European population. For quality control, SNPs with a call rate < 90% and P_{HWE} < 0.01 were excluded. A P-value after Bonferroni corrections of less than 3.57 × 10^{-3} (0.05/14) was regarded as statistically significant.

**Bioinformatics Analysis**

Several bioinformatics tools were utilized in this study. The Single Nucleotide Polymorphism database (dbSNP) was used for gene mapping. HaploReg4.1 was adopted to select the strongly linked SNPs and evaluate the potential biological significance for targeted SNPs. In addition, expression quantitative trait loci

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**TABLE 1** Demographic details of the subjects.

| Total samples | Case | Control |
|---------------|------|---------|
| 2581          | 2579 |         |
| Average age (age)(X ± SD) | 28.53 ± 14.22 | 31.95 ± 15.06 |
| Age arranged | 1–85 | 1–91    |
| Male          | 1355 (52.50%) | 1648 (63.90%) |
| Female        | 1226 (47.50%) | 931 (36.10%) |

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1. http://www.ncbi.nlm.nih.gov/snp
2. https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php
(eQTL) study data based on the GTEx database were adopted (GTEx Consortium, 2013).

RESULTS

Association Between SNPs and Vitiligo

In this study, 26 selected SNPs from 16 loci were genotyped in 2581 vitiligo patients and 2579 healthy controls. Twelve SNPs were eliminated for not meeting the inclusion criteria (call rate < 90%, \( P_{\text{HWE}} < 0.01 \)), leaving 14 SNPs for analysis. Four SNPs showed significant associations with vitiligo in the independent Chinese Han cohort, including rs3747517 \((P = 1.29 \times 10^{-3}, \text{OR} = 0.87, 95\% \text{CI}: 0.80–0.95)\) at 2q24.2, rs4807000 \((P = 7.78 \times 10^{-24}, \text{OR} = 0.66, 95\% \text{CI}: 0.61–0.71)\) and rs6510827 \((P = 3.65 \times 10^{-5}, \text{OR} = 1.19, 95\% \text{CI}: 1.09–1.29)\) at 19p13.3, and rs4822024 \((P = 6.37 \times 10^{-10}, \text{OR} = 0.67, 95\% \text{CI}: 0.58–0.76)\) at 22q13.2 (Table 3). However, no evidence of a significant association was observed for any other SNPs.

Functional Annotation via Bioinformatics Analysis

The positive vitiligo SNPs rs4807000 and rs6510827 are in the strong linkage disequilibrium (LD) region \((r^2 = 0.99)\) of 19p13.3 (Supplementary Figure 1), separately in 140 bp upstream and intronic region of TICAM1. According to Haploreg v4.1, these two SNPs are in the area of enhancer histone H3K4me1 in primary melanocyte cells and primary keratinocyte cells. The eQTL data extracted from GTEx show that they

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**Table 2** Information about the 32 SNPs in the GWAS and 1000 genome data.

| Chr | SNP | Position | Allele | Minor Allele Frequency | \( P \) | MAF\(_{\text{CEU}}\) | Origin |
|-----|-----|----------|--------|------------------------|--------|----------------|--------|
|     |     |          |        | Case                   |        | Control        |        |
| 1p36.23 | rs301807 | 8424763 | G/A | 0.1605 | 6.15 \times 10^{-1} | 0.54 | Reported |
| 1p36.23 | rs12745477 | 8204397 | A/G | 0.2432 | 2.28 \times 10^{-3} | 0.23 | Imputed |
| 2p16.3 | rs6544997 | 47617366 | A/G | 0.3917 | 1.65 \times 10^{-1} | 0.50 | Reported |
| 2p16.3 | rs72811506 | 47864584 | T/A | 0.1591 | 4.92 \times 10^{-3} | 0.01 | Imputed |
| 2q24.2 | rs3747517 | 162273214 | T/C | 0.3357 | 5.65 \times 10^{-1} | 0.74 | Reported |
| 2q24.2 | rs2300755 | 16202742 | A/G | 0.2781 | 2.56 \times 10^{-3} | 0.40 | Imputed |
| 3q13.33 | rs59374417 | 119585667 | A/C | 0.2634 | 5.51 \times 10^{-1} | 0.11 | Reported |
| 3q13.33 | rs16829716 | 119343558 | A/G | 0.0942 | 6.56 \times 10^{-3} | 0.09 | Imputed |
| 5q32 | rs251464 | 149816671 | G/C | 0.2249 | 1.63 \times 10^{-1} | 0.25 | Reported |
| 5q32 | rs9324834 | 149956876 | G/A | 0.0822 | 6.26 \times 10^{-3} | 0.12 | Imputed |
| 6p25.2 | rs78521699 | 2908357 | A/G | 0.2122 | 1.80 \times 10^{-1} | 0.13 | Reported |
| 6p25.2 | rs160666 | 2810535 | C/T | 0.2202 | 4.03 \times 10^{-3} | 0.82 | Imputed |
| 7p12.1 | rs10250629 | 50151757 | C/T | 0.2454 | 7.21 \times 10^{-2} | 0.43 | Reported |
| 7p12.1 | rs17633571 | 50133872 | T/C | 0.1842 | 1.85 \times 10^{-2} | 0.41 | Imputed |
| 8q24.22 | rs2687812 | 132918810 | A/T | 0.3669 | 2.85 \times 10^{-1} | 0.53 | Reported |
| 8q24.22 | rs1403486 | 132972870 | A/G | 0.2131 | 8.43 \times 10^{-3} | 0.62 | Reported |
| 11q21 | rs11021232 | 95567644 | T/C | 0.1031 | 4.49 \times 10^{-2} | 0.20 | Reported |
| 11q21 | rs16922181 | 95556687 | G/T | 0.1598 | 1.62 \times 10^{-2} | 0.12 | Imputed |
| 13q14.11 | rs35860234 | 42496070 | T/G | 0.1371 | 2.36 \times 10^{-4} | 0.29 | Reported |
| 13q14.11 | rs146191964 | 42520495 | T/A | 0.1348 | 1.52 \times 10^{-4} | 0.18 | Imputed |
| 17q25.3 | rs870355 | 78170115 | G/A | 0.4933 | 9.46 \times 10^{-1} | 0.41 | Reported |
| 17q25.3 | rs749885 | 78270727 | G/A | 0.3335 | 5.00 \times 10^{-3} | 0.47 | Imputed |
| 18q12.31 | rs10503019 | 57787145 | G/A | 0.0927 | 2.80 \times 10^{-1} | 0.23 | Reported |
| 18q12.31 | rs7226959 | 57764310 | T/C | 0.1200 | 7.58 \times 10^{-4} | 0.30 | Reported |
| 19p13.3 | rs6510827 | 4830616 | C/T | 0.4382 | 8.70 \times 10^{-3} | 0.62 | Reported |
| 19p13.3 | rs4807000 | 4831866 | G/A | 0.4396 | 6.32 \times 10^{-3} | 0.62 | Reported |
| 19q13.3 | rs2304206 | 49665614 | G/A | 0.1529 | 1.07 \times 10^{-2} | 0.26 | Reported |
| 19q13.3 | rs12986313 | 49455384 | C/A | 0.2168 | 1.16 \times 10^{-2} | 0.40 | Reported |
| 21q22.11 | rs2833807 | 32008727 | G/A | 0.2672 | 5.25 \times 10^{-2} | 0.23 | Reported |
| 21q22.11 | rs9305469 | 31779113 | T/G | 0.1584 | 4.19 \times 10^{-3} | 0.85 | Imputed |
| 22q13.2 | rs4822024 | 41361643 | A/C | 0.0880 | 6.62 \times 10^{-2} | 0.24 | Reported |
| 22q13.2 | rs5758961 | 41456728 | A/C | 0.4302 | 1.50 \times 10^{-3} | 0.00 | Imputed |

Chr: chromosome; SNP: single-nucleotide polymorphism; MAF\(_{\text{CEU}}\): minor allele frequencies of SNPs in European population from 1000 Genome Project phase 1 database.

*Positions are based on human genome version 38 (hg38). Minor allele/major allele.*
TABLE 3 | Summary of association results of 14 SNPs in 11 loci between cases and controls.

| SNP Position | Allele Frequencies | Case | Control |
|--------------|-------------------|------|---------|
| rs12745477   | A/G               |      |         |
| rs6544997    | A/G               |      |         |
| rs2300755    | A/G               |      |         |
| rs160669     | C/T               |      |         |
| rs4822024    | C/T               |      |         |

Positions are based on human genome version 38 (hg38).

Minor allele/major allele frequencies were considered to be statistically significant (with Bonferroni corrections); P-values of less than 3.57 × 10^{-8} are reported by vitiligo GWASs in European-derived whites; “–” indicates no positive results.

DISCUSSION

Vitiligo is a common depigmenting skin disorder influenced by genetic and environmental factors. Our team conducted a series of genetic studies of vitiligo in the Chinese Han population. A GWAS of vitiligo was carried out in 2010. The 34 most promising SNPs in the MHC region were replicated, and one new risk locus was identified at 6q27, which contains three genes, RNASET2, FGFR1OP, and CCR6 (Quan et al., 2010). In 2013, the previous vitiligo GWAS was extended with a two-stage replication study, and 50 SNPs at 44 loci showing a suggestive association (P_{initial} < 1 × 10^{-4} in GWAS) were selected for replication testing, which identified three susceptibility loci (12q13.2, 11q23.3, and 10q22.1) for vitiligo (Tang et al., 2013). In 2015, 12q13.2 was verified to be significantly associated with vitiligo accompanying immune-related diseases by testing 10 immune susceptibility SNPs (Li et al., 2015). In 2017, two newly identified SNPs (rs613791 and rs523604) showed independent signals in the associated locus 11q23.3 for vitiligo (Zhao et al., 2017). In 2019, 3q29 was demonstrated to be associated with vitiligo after genotyping 14 reported loci identified from a meta-analysis of GWAS in European-derived whites (Tang et al., 2019). In this study, 16 reported susceptible loci (1 × 10^{-3} > P_{initial} > 1 × 10^{-4} in our GWAS, located in non-MHC regions) in European-derived whites by GWAS were first tested to be associated with vitiligo in the Chinese Han population (Table 2), which revealed the strong association of 2q42.2 (rs3747517), 19p13.3 (rs4807000 and rs6518027), and 22q13.2 (rs4822024) with vitiligo.

SNP rs3747517 in 2q42.2 is a missense variant of IFIH1 encoding the IFIH1 protein, which can activate innate immune reaction to bind to damage-associated molecular patterns (Looney et al., 2015). Dysfunction of IFIH1 impairs the activation of downstream innate immune responses (Jin et al., 2017). SNPs rs4807000 and rs6518027 in 19p13.3 are located separately in 140 bp upstream and the intron of TICAM1 with significant
LD ($r^2 = 0.99$). TICAMI regulates the innate immune reaction to viruses by inducing pattern recognition receptor–mediated IFN production (Seya et al., 2009). SNP rs4822024 is located 6 kb upstream of TEF, which encodes the TEF protein, and is associated with the expression level of TEF in blood (GTEx Consortium, 2015). TEF is a member of the proline and acidic amino-acid-rich basic leucine zipper (PAR bZip) transcription factor family, which plays a pivotal role in regulating circadian rhythm. The above loci may be involved in the pathogenesis of vitiligo through immune or other mechanisms.

The current findings revealed four SNPs in three loci that contribute to vitiligo susceptibility in Chinese Han individuals. However, we were unable to obtain evidence of an association for the other 10 SNPs. Aside from these 14 SNPs, 6 SNPs failed in the assay design, and 12 SNPs were eliminated for not meeting the quality-control criteria. The MAFs of these SNPs exhibited some difference between the Chinese Han (CHB) and European (CEU) cohorts (Table 2), and the correlation identified in the European population may not be well present in this study. Power analysis showed that the sample of 2581 patients and 2579 controls provided insufficient statistical power (<80%) for these negative SNPs except for rs59374417 in 3q13.33, which was not sufficient for detecting an association (Table 3), so larger sample sizes are needed in future studies. In addition, the difference in association evidence for rs59374417 between the Chinese Han and European-derived whites may implicate a genetic heterogeneity of vitiligo susceptibility between ethnic populations.

In this study, we tested the association of 16 susceptible SNPs for vitiligo identified in European-derived whites and the respective top imputed SNPs of candidate regions in a Chinese Han population. The negative results only indicated that these SNPs, not their LD region, were not related to vitiligo in the Chinese Han population. The differences in LD structure around the causal variants might result in distinct observations of associations in different populations. A more in-depth comparison of the LD structures in the candidate regions in Chinese Han versus European populations is necessary, either by targeted sequencing or fine genotyping in future studies.

In conclusion, the strong association of 2q24.2 (rs3747517), 19p13.3 (rs4807000 and rs6510827), and 22q13.2 (rs4822024) with the risk of vitiligo was demonstrated in a Chinese Han population. The differences in LD structure between the Chinese Han and European-derived whites may be involved in the pathogenesis of vitiligo through immune or other mechanisms.

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**DATA AVAILABILITY STATEMENT**

All datasets generated for this study are included in the article/Supplementary Material.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Ethics Committee of the Anhui Medical University. Written informed consent to participate in this study was provided by the participants.

**AUTHOR CONTRIBUTIONS**

F-LX designed and supervised the study. LC wrote and revised the manuscript. X-YC helped to analyze the data. X-FT helped to select the SNPs. BL and HC enrolled the patients. LC, JiZ, MW-W, and JuZ conducted the experiments. X-DZ processed the data and performed statistical analysis. F-SZ and PL helped with genotyping. All authors contributed to the article and approved the submitted version.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2020.542275/full#supplementary-material
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Conflict of Interest: 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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