An in-depth analysis reveals two new genetic variants on 22q11.2 associated with vitiligo in the Chinese Han population

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Abstract

Background Vitiligo is a complex disease in which patchy depigmentation is the result of an autoimmune-induced loss of melanocytes in affected regions. On the basis of a genome-wide linkage analysis of vitiligo in the Chinese Han population, we previously showed significant evidence of a linkage between 22q12 and vitiligo. Our aim in the current study was to identify vitiligo susceptibility variants within an expanded region of the 22q12 locus.

Methods and results An in-depth analysis of the expanded region of the 22q12 locus was performed by imputation using a large GWAS dataset consisting of 1117 cases and 1701 controls. Eight nominal SNPs were selected and genotyped in an independent cohort of Chinese Han individuals (2069 patients and 1370 control individuals) by using the Sequenom MassArray iPLEX1 system. The data were analyzed with PLINK 1.07 software. The C allele of rs730669 located in ZDHHC8/RTN4R showed a strong association with vitiligo (P = 3.25 × 10⁻⁸, OR = 0.81). The C allele of rs4820338 located in VPREB1 and the A allele of rs2051582 (a SNP reported in our previous study) located in IL2RB showed a suggestive association with vitiligo (P = 1.04 × 10⁻⁵, OR = 0.86; P = 1.78 × 10⁻⁶, OR = 1.27). The three identified SNPs showed independent associations with vitiligo in a conditional logistic regression analysis (all P < 1.0 × 10⁻⁵; all D' < 0.05 and r² < 1.0 × 10⁻⁴).

Conclusions The study reveals that two novel variants rs730669 (ZDHHC8/RTN4R) and rs4820338 (VPREB1) on 22q11.2 might confer susceptibility to vitiligo and affect disease subphenotypes. The presence of multiple independent variants emphasizes their important roles in the genetic pathogenesis of disease.

Keywords Vitiligo · Genetic · Association study · Susceptibility loci · SNPs

Background

Vitiligo is a complex disease in which patchy depigmentation is the result of an autoimmune-induced loss of melanocytes in affected regions. It is characterized by the loss of epidermal melanocytes leading to localized or generalized depigmentation or loss of plaque in the epidermis and mucous membranes [1]. Epidemiological surveys show that the incidence of vitiligo is approximately 0.5–1% [2]. Its underlying pathobiology may involve genetics, immunity and environment. Linkage and association studies of multiethnic populations revealed some susceptibility/candidate genes on chromosomes (such as, 4q13-q21, 1p31, and 17p13) [3, 4], which provided strong support for genetic loci of vitiligo. Notably, linkage evidence for a vitiligo locus on 22q12 was revealed in our previous genome-wide linkage analysis of the Chinese Han population [5]. To date, sixty genetic loci associated with disease susceptibility have been identified by several genome-wide association studies (GWAS) of vitiligo in Caucasians and Chinese individuals [6–12], most of which harbor genes involved in the regulation of biological functions (melanocytes, apoptosis and immunity). These findings further emphasize in a common model of melanocyte autoimmune pathogenesis [13].
To identify the susceptibility variants in the identified vitiligo locus on 22q12 by linkage analysis [5], a fine imputation analysis of the 22q12 locus was performed using our previous obtained GWAS dataset [11, 12], and further validation analysis was performed in an independent cohort of the Chinese Han population, which provided additional information on the locus implicated in the genetic pathogenesis of vitiligo.

Materials and methods

Subjects

In total, the data from our previous vitiligo GWAS of 1117 vitiligo patients and 1701 controls were used [11, 12], while 2069 cases and 1370 controls were recruited for a replication analysis in this study (Table 1). The data on all individuals (Chinese Han population) were from multiple hospitals in China. After written informed consent was obtained from all individuals, blood samples and clinical and demographic information (obtained through a previously designed questionnaire) were collected from the previous patients and controls group individuals. Based on the principles of the Declaration of Helsinki, this study was approved by the Institutional Ethical Committee of Anhui Medical University. According to the diagnostic criteria of the Vitiligo European Task Force [14], the clinical diagnosis of all patients was confirmed by at least two dermatologists. All control subjects were healthy individuals without vitiligo, familial history (including first-, second- and third-degree relatives) of vitiligo, or any other autoimmune diseases or systemic disorders. Common factors (ethnicity, age and sex) were matched in the case and control groups. According to the manufacturer’s instructions, genomic DNA was extracted from peripheral blood lymphocytes by using a QIAamp DNA Blood kit (Qiagen, Valencia, CA, USA) and diluted to working concentrations of 20–25 ng/ml for the replication study.

SNP selection for the replication analysis

Based on our previous vitiligo GWAS [11, 12] and the reference panel of the 1000 Genomes Project (Mar. 2012 release) [15], imputation for an expanded region of the 22q12 locus (expansion of 5 Mb upstream and downstream for this locus, chr 22: 20,000,000–42,000,000, hg19) was performed on samples from 1117 vitiligo patients and 1701 controls by using Impute v2.0 software [16]. The following conditions led to exclusion from further analysis: (1) SNPs with low imputation confidence (INFO score ≤ 0.5); (2) significant Hardy–Weinberg disequilibrium (P < 0.05); (3) a MAF < 1%; (4) a low call rate < 95%. Finally, 8 SNPs at the region showed association significance (P_initial < 0.01) in our vitiligo GWAS and were therefore selected for this replication study.

Genotyping analysis of the replication study

The detection primers for the 8 SNPs were designed using the MassARRAY Assay Design 3.0 software (Sequenom). Eight SNPs were genotyped by using the Sequenom MassArray iPLEX1 system (the Key Laboratory of Dermatology, Ministry of Education, China). The details were described in previous studies [11, 12].

Statistical analysis

The association of each SNP with disease was tested in the discovery and replication stages in patient and control samples by using PLINK version 1.07 software [17]. Principal component analysis was used to assess population outliers and stratification in the dataset [11]. GWAS and replication association analyses were carried out by using the Cochran-Armitage trend test. Conditional logistic regression analysis was performed to identify any independent effects, and this test was carried out with the SNPTEST (V2) program. The regional plot of the association result was generated by using LocusZoom which was based on the information of JPT and CHB populations from hg19/HapMap Phase II. The level of associated significance was assigned at P values less than 5.0 × 10^{-8} (the criterion for genome-wide significance).

Table 1 Summary information of vitiligo patients and controls

| Analysis  | Cases      | Controls       |
|-----------|------------|----------------|
|           | Sample size| Sample size    |
|           | Mean age (s.d.)| Mean age (s.d.)|
|           | Male (%)/female (%)| Male (%)/female (%)|
| GWAS      | 1117       | 1701           |
| Replication| 2069      | 1370           |
| Total     | 3186       | 3071           |

GWAS genome-wide association study
Subphenotype analyses of the associated SNPs were also performed according to clinical features which included sex, familial history, age of onset (early onset ≤ 20 years and late onset > 20 years), clinical classification (non segmental and segmental) and autoimmune disease involvement (such as, systemic lupus erythematosus, alopecia areata, thyroid disease, rheumatoid arthritis, myasthenia gravis, and scleroderma), P values of less than 5.0×10^{-2} were considered to be statistically significant.

**Bioinformatics analysis**

Several bioinformatics tools were utilized in this study. HaploReg4.1 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) was used to select the strongly linked SNPs and evaluate the potential biological significance of the targeted SNPs. Single Nucleotide Polymorphism database (dbSNP) was used for gene mapping (http://ncbi.nlm.nih.gov/snp). Based on publicly available data (GTEx, http://gtexportal.org/home/), the expression quantitative trait loci (eQTL) analysis was used to evaluate whether the significant SNPs modified the expression and regulation of genes in normal tissues.

**Results**

**Association between SNPs and vitiligo**

The call rates for the selected 8 SNPs were > 95% and there were no significant deviations from the Hardy–Weinberg equilibrium (P > 0.05) in the control subjects in this replication study. Table 2 shows the association analysis for the 8 SNPs on 22q12 for the patients and controls. In this replication study, the minor alleles of SNPs rs730669, rs4820338 and rs2051582 were weakly associated with vitiligo (P = 1.34×10^{-4}, OR = 0.82, 95% CI: 0.74–0.91; P = 7.28×10^{-3}, OR = 0.88, 95% CI: 0.80–0.97; P = 4.54×10^{-4}, OR = 1.27, 95% CI: 1.11–1.45). The combined analyses of the GWAS dataset and replication stages showed a strong association between vitiligo and the SNP rs730669 (P = 3.25×10^{-8}, OR = 0.81, 95% CI: 0.75–0.87, reaching genome-wide significance) and suggestive associations between vitiligo and the SNPs rs4820338 (P = 1.04×10^{-5}, OR = 0.86, 95% CI: 0.80–0.92) and rs2051582 (P = 1.78×10^{-6}, OR = 1.27, 95% CI: 1.15–1.40, a variant reported in our previous study [12]).

**Conditional logistic regression analysis**

To further evaluate the genetic effect and independent associated signal among these three significant SNPs, we performed a conditional logistic regression analysis by controlling for the genetic effect of the associated SNPs (Table 3). By controlling for the reported SNP rs2051582, we found that the two newly identified SNPs (rs730669 and rs4820338) also showed significant associations with vitiligo (P = 4.15×10^{-8} and P = 9.13×10^{-6}). Controlling for the rs730669 or rs4820338 SNP, we found that the other two SNPs (rs2051582 and rs4820338; rs730669 and rs2051582) also exhibited significant associations with vitiligo (P = 2.17×10^{-6} and P = 5.12×10^{-6}; P = 7.87×10^{-9} and P = 3.07×10^{-5}). Among the three SNPs, the paired SNPs were not correlated with each other in the current study population (all D’ < 0.05 and r^2 < 1.0×10^{-4}).

**Genotype and subphenotype analyses**

Analyses of genotype–phenotype (age of onset, sex, familial history, clinical classification, concomitant autoimmune diseases and Koebner phenomenon) of the two SNPs were also performed. For the SNP rs4820338, the frequency of genotypes (P = 2.30×10^{-2}) and alleles (P = 7.78×10^{-3}, OR = 0.87, 95% CI, 0.79–0.97) was significantly different between patients with early onset and late onset (Table 4). However, no significant difference in the subgroups was observed for the associated SNP rs730669 (Table 5).

**Functional annotation and bioinformatics analysis**

The two associated SNPs rs730669 and rs4820338 are both located on 22q11.2 (Fig. 1). Rs730669 is located 66 kb downstream of Zinc finger DHHC-type containing 8 (ZDHHC8) and 27 kb downstream of reticulon 4 receptor (RTN4R), rs4820338 is located in 78 kb upstream of V-set pre-B cell surrogate light chain 1 (VPREB1). The eQTL analysis using the GTEx database showed that only the SNP rs730669 was significantly associated with the expression level of ZDHHC8 in cultured fibroblasts cells (P = 9.10×10^{-6}).

**Discussion**

To explore the contribution of genetic variation of 22q12 previously identified by linkage analysis in vitiligo, we performed further imputation analysis on the expanded region of this locus using the discovery dataset and then replicated this analysis with data from an independent cohort of the Chinese Han population. We thus identified two novel genetic variants (rs730669 and rs4820338) on 22q11.2 that were associated with vitiligo, and confirmed that our previously reported variant (rs2051582) is on 22q12.3 [12]. Several susceptible variants on 22q13.1 were also reported to be associated with vitiligo in the Caucasian population [6]. Conditional logistic regression analysis showed...
Table 2  Association evidence for 8 SNPs in the GWAS, the replication study, and the combined analysis

| SNP       | Chr_pos | Gene          | MA  | GWAS                     | Replication study | Combined Chinese Han | P     | OR (95% CI)    | Call rate | MA  | OR (95% CI)    | Call rate | P     | OR (95% CI)    | Call rate |
|-----------|---------|---------------|-----|--------------------------|-------------------|----------------------|-------|---------------|-----------|-----|---------------|-----------|-------|---------------|-----------|
| rs730669  | 20,214,431 | ZDHHC8/RTN4R  | C   | 0.32 0.37                | 0.81 (0.73–0.91)  | 2.99E-04 1.00       | 0.32 0.36 | 0.82 (0.74–0.91) | 1.34E-04 0.99 | 0.81 (0.75–0.87) | 3.25E-08 2.03E-01 |
| rs5750483 | 22,080,038 | IGLV8-61      | A   | 0.23 0.27                | 0.82 (0.73–0.93)  | 1.94E-03 1.00       | 0.25 0.25 | 0.96 (0.86–1.08) | 5.23E-01 0.95 | 0.89 (0.82–0.97) | 5.87E-03 5.52E-02 |
| rs4820338 | 22,166,665 | VPREB1        | C   | 0.44 0.48                | 0.83 (0.75–0.93)  | 9.18E-04 0.99       | 0.44 0.48 | 0.88 (0.80–0.97) | 7.28E-03 0.99 | 0.86 (0.80–0.92) | 1.04E-05 7.43E-02 |
| rs490362  | 27,335,509 | LOC102724900  | C   | 0.20 0.17                | 1.27 (1.11–1.46)  | 6.13E-04 1.00       | 0.18 0.18 | 1.03 (0.91–1.17) | 6.35E-01 0.99 | 1.13 (1.03–1.24) | 1.08E-02 2.10E-01 |
| rs133937  | 32,999,866 | SYN3          | C   | 0.21 0.25                | 0.83 (0.73–0.95)  | 5.03E-03 1.00       | 0.24 0.25 | 0.96 (0.86–1.08) | 4.91E-01 0.99 | 0.92 (0.85–1.00) | 4.44E-02 9.85E-01 |
| rs2051582 | 37,162,316 | IL2RB         | A   | 0.17 0.14                | 1.24 (1.07–1.45)  | 4.26E-03 1.00       | 0.17 0.14 | 1.27 (1.11–1.45) | 4.54E-04 0.99 | 1.27 (1.15–1.40) | 1.78E-06 6.29E-01 |
| rs576587  | 37,282,163 | CYTH4         | T   | 0.15 0.18                | 0.79 (0.68–0.91)  | 1.46E-03 1.00       | 0.17 0.16 | 1.04 (0.91–1.19) | 5.50E-01 0.97 | 0.92 (0.84–1.01) | 8.52E-02 4.53E-01 |
| rs4820287 | 37,394,071 | ELFN2         | C   | 0.24 0.28                | 0.81 (0.71–0.91)  | 6.65E-04 0.99       | 0.26 0.26 | 1.04 (0.93–1.16) | 5.40E-01 0.99 | 0.92 (0.85–1.00) | 4.66E-02 1.04E-01 |

GWAS genome-wide association study, SNP single-nucleotide polymorphism, Chr_pos chromosome position, MA minor allele, MAF minor allele frequency, OR odds ratio, CI confidence interval

PHWE = P value for the test of Hardy–Weinberg equilibrium
that the associations of the other two SNPs still appeared ($P < 1.0 \times 10^{-5}$) after controlling for the genetic effect of each SNP at this locus, which indicated that the associations of these three SNPs were independent of each other. Three significant SNPs (rs730669, rs4820338 and rs2051582) were in very weak linkage disequilibrium (LD) with each other (all pairwise $D' < 0.05$ and $r^2 < 1.0 \times 10^{-4}$ in current sample set). Conditional analysis has been used as an effective method to identify additional association signals by conditioning for the genetic effect of an associated SNP at a locus [18]. In the current conditional logistic regression analysis, the $P$ values for the other SNP effects indicate weak decreases or changes due to a reduction in sample size resulting from the removal of the individuals who carried the allele of the controlled SNP.

The most strongly associated SNP rs730669 on 22q11.2 is located in a LD block that includes multiple genes (Fig. 1); however, most of the functions for these genes are largely unknown. The SNP rs730669 on 22q11.2 is located in an intergenic region between ZDHHC8 and RTN4R. ZDHHC8

### Table 3 The results of conditional regression analysis

| SNP       | $r^2$         | $D'$        | Condition on SNP | $P$     | OR   | 95% CI |
|-----------|---------------|-------------|------------------|---------|------|--------|
| rs730669  | 4.51E-04      | 3.17E-02    | rs2051582        | 4.15E-08| 0.81 | 0.75–0.87|
| rs4820338 | 9.13E-06      |             | rs730669         | 2.17E-06| 1.27 | 1.15–1.40|
| rs2051582 | 1.13E-05      | 7.25E-03    | rs4820338        | 5.12E-06| 0.84 | 0.78–0.91|
| rs730669  | 8.28E-05      | 2.93E-02    | rs4820338        | 7.87E-09| 0.80 | 0.74–0.86|
| rs2051582 | 3.07E-06      |             |                  | 1.26    |      | 1.15–1.40|

$SNP$ single-nucleotide polymorphism, $OR$ odds ratio, $CI$ confidence interval

### Table 4 Distribution of genotypes and alleles for SNP rs4820338 located in VPREB1 for subgroups

| Subphenotypes | Genotype frequency (%) | Allele frequency (%) |
|---------------|------------------------|----------------------|
|               | CC  | CG  | GG  | $P^a$ | C   | G   | $P^b$ | OR(95%CI)$^c$ |
| Age at onset  |     |     |     |       |     |     |       |              |
| Early onset $\leq 20$ years (n = 1703) | 291 (17.09) | 873 (51.26) | 539 (31.65) | 2.30E-02 | 1455 (42.72) | 1951 (57.28) | 7.78E-03 | 0.87 (0.79–0.97) |
| Late onset $> 20$ years (n = 1447)  | 286 (19.77) | 761 (52.59) | 400 (27.64)  |       | 1333 (46.06) | 1561 (53.94) |       |              |
| Sex           |     |     |     |       |     |     |       |              |
| Male (n = 1714) | 320 (18.67) | 884 (51.58) | 510 (29.75)  | 0.85  | 1524 (44.46) | 1904 (55.54) | 0.72    | 1.02 (0.92–1.13) |
| Female (n = 1436) | 257 (17.90) | 750 (52.23) | 429 (29.78)  |       | 1264 (44.01) | 1608 (55.99) |       |              |
| Familial history |     |     |     |       |     |     |       |              |
| Positive (n = 414) | 89 (21.50) | 213 (51.45) | 112 (27.05)  | 0.15  | 391 (47.22) | 437 (52.78)  | 0.07    | 1.15 (0.99–1.33) |
| Negative (2736)  | 488 (17.84) | 1421 (51.94) | 827 (30.22)  |       | 2397 (43.80) | 3075 (56.20) |       |              |
| Clinical classification |     |     |     |       |     |     |       |              |
| Non segmental (n = 2297) | 439 (19.11) | 1173 (51.07) | 685 (29.82)  | 0.14  | 2051 (44.65) | 2543 (55.35) | 0.31    | 1.06 (0.95–1.19) |
| Segmental (n = 853) | 138 (16.18) | 461 (54.04) | 254 (29.78)  |       | 737 (43.20) | 969 (56.80)  |       |              |
| Autoimmune disease involvement |     |     |     |       |     |     |       |              |
| Positive (n = 92) | 20 (21.74) | 49 (53.26) | 23 (25.00)  | 0.50  | 89 (48.37) | 95 (51.63)  | 0.25    | 1.19 (0.88–1.59) |
| Negative (n = 3058) | 557 (18.21) | 1585 (51.83) | 916 (29.96)  |       | 2699 (44.13) | 3417 (55.87) |       |              |
| Koebner phenomenon |     |     |     |       |     |     |       |              |
| Positive (n = 236) | 51 (21.61) | 121 (51.27) | 64 (27.12)  | 0.34  | 223 (47.25) | 249 (52.75) | 0.17    | 1.14 (0.94–1.38) |
| Negative (n = 2914) | 526 (18.05) | 1513 (51.92) | 875 (30.03) |       | 2565 (44.01) | 3263 (55.99) |       |              |

C is the minor allele and G is the risk allele  
$OR$ odds ratio, $CI$ confidence interval  
$^a$For genotype using a $2 \times 3$ contingency table  
$^b$For allele using a $2 \times 2$ contingency table  
$^c$using G allele as reference
contains 11 exons and encodes a palmitoyltransferase that shares a conserved cysteine-rich signature catalytic domain (belonging to a 23-member family of enzymes). Genetic variants of ZDHHC8 have been reported to be associated with schizophrenia [19, 20], epilepsy [21] and total anomalous pulmonary venous connection [22]. The eQTL data showed that the SNP rs730669 was associated with a significant effect on the expression level of ZDHHC8 in cultured fibroblasts cells. Fibroblasts might affect melanocyte or epidermal pigmentation function by deregulating the secretion of skin aging-associated proteins and various cytokines from fibroblasts, which favors pigmentation loss and progression of vitiligo [23, 24]. This functional eQTL result suggests that the genetic association effect of rs730669 may impact the transcriptional regulation and gene expression of ZDHHC8 in dermal fibroblasts through noncoding genetic regulatory effects. It is well known that catecholamines (dopamine) acts as neurotransmitter that play important roles in the pathogenesis of vitiligo [25]. It is likely that the dopamine pathway directly affects melanogenesis through the melanocortin pathway. ZDHHC8 can interact with the D2 dopamine receptor (D2R), which might influence the palmitoylation status of D2R [26]. A difference in the mRNA expression of the D2R gene has observed in the skin of patients with vitiligo [27]. These results indicate that ZDHHC8 might participate in the pathogenetic mechanisms of vitiligo through the above mentioned biological pathway.

The candidate gene RTN4R [also known as neurite outgrowth inhibitor 66 (NgR)] contains 2 exons and encodes a glycosylphosphatidylinositol (GPI)-linked protein that plays an important role in the inhibition of myelin-mediated axonal growth by interacting with NgR [28]. The genetic variants of RTN4R have been reported to be associated with the anterior limb of the internal capsule and psychosis in 22q11.2 deletion syndrome [29, 30]. Some studies have also shown that the distinct expression of NgR in multiple immunological cells (monocytes, dendritic cells and T/B cells) influences the subsequent regulation of the adhesion or stimulation of these cells, which plays an important role in the pathogenesis of neuroinflammatory diseases such as multiple sclerosis [31, 32]. Together, these results further implicate neural-inflammation mediators in vitiligo pathogenesis [33].

The associated SNP rs4820338 on 22q11.2 is located in a LD block that only contains VPREB1 (Fig. 1). VPREB1 contains 2 exons and encodes a protein that belongs to the immunoglobulin superfamily. It is expressed selectively at the early stages of immune B cell development, which may

Table 5 Distribution of genotypes and alleles for SNP rs730669 located in ZDHHC8/RTN4R for subgroups

| Genotype frequency (%) | Allele frequency (%) |
|------------------------|----------------------|
| Subphenotypes          | CC  | CT  | TT  | P^ | C  | T  | P^ | OR(95%CI)^c |
| Age at onset            |     |     |     |    |    |    |    |     |
| Early onset < = 20 years (n = 1717) | 178 (10.37) | 748 (43.36) | 791 (46.07) | 0.99 | 1104 (32.15) | 2330 (67.85) | 0.89 | 1.01 (0.91–1.12) |
| Late onset > 20 years (n = 1454) | 148 (10.18) | 634 (43.60) | 672 (46.22) | 0.38 | 930 (31.98) | 1978 (68.02) |     |     |
| Sex                     |     |     |     |    |    |    |    |     |
| Male (n = 1732)         | 181 (10.45) | 731 (42.21) | 820 (47.34) | 0.23 | 1093 (31.55) | 2371 (68.45) | 0.33 | 0.95 (0.85–1.06) |
| Female (n = 1439)       | 145 (10.08) | 651 (45.24) | 643 (44.68) | 0.31 | 941 (32.70) | 1937 (67.30) |     |     |
| Familial history         |     |     |     |    |    |    |    |     |
| Positive (n = 417)      | 49 (11.75) | 178 (42.69) | 190 (45.56) | 0.57 | 276 (33.09) | 558 (66.91) | 0.50 | 1.06 (0.90–1.23) |
| Negative (2754)         | 277 (10.06) | 1204 (43.72) | 1273 (46.22) | 0.01 | 1758 (31.92) | 3750 (68.08) |     |     |
| Clinical classification  |     |     |     |    |    |    |    |     |
| Non segmental (n = 2310) | 250 (10.82) | 1006 (43.55) | 1054 (45.63) | 0.23 | 1506 (32.60) | 3114 (67.40) | 0.14 | 1.09 (0.97–1.23) |
| Segmental (n = 861)     | 76 (8.83) | 376 (43.67) | 409 (47.50) | 0.31 | 528 (30.66) | 1194 (69.34) |     |     |
| Autoimmune disease involvement |     |     |     |    |    |    |    |     |
| Positive (n = 94)       | 12 (12.77) | 35 (37.23) | 47 (50.00) | 0.41 | 59 (31.38) | 129 (68.62) | 0.84 | 0.97 (0.71–1.32) |
| Negative (n = 3077)     | 314 (10.20) | 1347 (43.78) | 1416 (46.02) | 0.23 | 1975 (32.09) | 4179 (67.91) |     |     |
| Koebner phenomenon     |     |     |     |    |    |    |    |     |
| Positive (n = 235)      | 20 (8.51) | 109 (46.38) | 106 (45.11) | 0.53 | 149 (31.70) | 321 (68.30) | 0.86 | 0.98 (0.80–1.20) |
| Negative (n = 2936)     | 306 (10.42) | 1273 (43.36) | 1357 (46.22) | 0.23 | 1885 (32.10) | 3987 (67.90) |     |     |

C is the minor allele and T is the risk allele

OR odds ratio, CI confidence interval

^a For genotype using a 2 × 3 contingency table

^b For allele using a 2 × 2 contingency table

^c Using T allele as reference

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Fig. 1 Regional association plot for the associated SNPs rs730669 and rs4820338 at 22q11.2 region. SNP is plotted by chromosomal position (GRCh38/hg19; x axis) and association with vitiligo from current study (−log10 P-value; y axis). Estimated recombination rates (based on the combined CHB and JPT samples from the HapMap project) were plotted in light blue. Genes are indicated in the lower panel of the plot. (Color figure online)
indicate that it regulates the level of Ig gene rearrangements in immune B cell differentiation. These findings showed that transcriptional and metabolic pre-B cell receptor-mediated ingredients play important roles in the pathogenesis of autoimmune diseases [34]. The deletion of VPREB1 has been reported to be associated with chronic autoimmune arthritis [35], rheumatoid arthritis [36], and acute lymphoblastic leukemia [37, 38]. Lymphoid immune surveillance contributes to vitiligo lesions and results in lesion extension by triggering antigen-specific T and B cell responses, supporting the involvement of the immune response in disease development [39]. The biological function of melanin-concentrating hormone receptor 1 (McHR1) is associated with a B-cell autoantigen located on the membrane of melanocytes, which blocks the response of the receptor to McH [40]. Reaction against multiple epitopes on McHR1 has been reported in vitiligo [41, 42]. Therefore, it is speculated that proteins abnormally encoded by VPREB1 might be involved in a variety of immunobiological pathways through the interaction between McHR1 and pre-B cell receptor-mediated ingredients, which further indicates that genetic and immune factors play important roles in the pathogenesis of vitiligo.

Studies showed that several genetic variants rs11966200 and rs145954018 in MHC region were associated with early onset vitiligo in Chinese and Caucasian populations [43, 44]. In the current study, early onset vitiligo was specifically associated with the SNP rs4820338. The minor allele C conferred protection against early disease onset. Similar stratified analysis also revealed that the tumor necrosis factor alpha (TNF-α) and discoidin domain receptor tyrosine kinase 1 (DDRI) genes polymorphisms allele conferred protection against early vitiligo [45, 46], which might contribute substantially to the potential immunological functions and heritability difference between the early-onset and late-onset subgroups. The current result provides a helpful clue indicating that immune factors might play key roles in the development of some clinical subtypes of disease. For the associated SNP rs730669, no association with disease phenotype was identified in the stratified analysis because different genes might contribute to different disease subphenotypes, which also explains the reason that complex genetic and environmental factors are involved in the pathogenesis of this disease.

This study revealed two novel independent variants, rs730669 (ZDHHC8/RTN4R) and rs4820338 (VPREB1), on 22q11.2 that contribute to vitiligo susceptibility, and thus emphasized that the related neuroimmunology genes play important roles in the pathogenetic mechanisms of this disease. The current findings provide a direction for functional studies of candidate genes at this locus in the future. It is still necessary to identify causal gene(s) by targeted sequencing and functional studies.

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Author contributions FX and XT designed research; HC, LC, and BL were responsible for sample selection and genotyping; MC and XZ undertook data processing, statistical analysis and bioinformatics investigations; XT wrote the paper.

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Declarations

Conflict of interest All authors have no conflicts of interest to declare.

Ethical approval This study was approved by the Institutional Ethical Committee of Anhui Medical University.

Consent to participate Respect for participants and Informed consent to participate in this research.

Consent to publish All authors agree to publication in this Journal and it is not under consideration for publication elsewhere.

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