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ORIGINAL ARTICLE

Mediation roles of neutrophils and high-density lipoprotein (HDL) on the relationship between HLA-DQB1 and rosacea

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ABSTRACT

Background: Though the previous genome-wide association studies found the association between *HLA* alleles and rosacea in the European populations, the data is lacking among the Asians. Moreover, neutrophils are important in the immune-related mechanism of rosacea, and dyslipidemia is closely related to rosacea. We aimed to explore the association between *HLA* genes and rosacea in Chinese rosacea patients, as well as the mediation effect of neutrophils, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) on the relationship between *HLA* genes and rosacea.

Methods: A total of 249 rosacea and 150 controls were ranked by the international investigator global rosacea severity scores. *HLA* genes, neutrophils, HDL, and LDL were detected. And their mediation effects on the relationship between *HLA* and rosacea risk or severity were analysed.

Results: *HLA-DQB1*03:03* allele (OR = 41.89, 95% CI: 9.80 ~ 179.09, $p = 4.7*10^{-7}$), *HLA-DQB1*04:02* allele (OR = 0.16, 95% CI: 0.03 ~ 0.81, $p = 0.026$) and *HLA-DQB1*03:03/05:02* genotype (OR = 5.57, 95% CI: 1.13 ~ 27.52, $p = 0.0351$) were significantly associated with rosacea. Moreover, *HLA-DQB1*03:03* allele (b = 1.434, SE = 0.217, $p = 2.0*10^{-10}$), *HLA-DQB1*05:01* allele (b = 0.894, SE = 0.33520, $p = 0.008$) and *HLA-DQB1*03:03/06:01* genotype (b = 0.998, SE = 0.472, $p = 0.040$) were positively associated with rosacea severity. Furthermore, we found both neutrophils and HDL, instead of LDL, have mediation effects on the relationship between *HLA-DQB1*03:03* and risk or severity of rosacea.

Conclusions: We discovered novel susceptible *HLA* alleles for rosacea in the Chinese population, and disclosed the mediation effect of neutrophils and HDL on the relationship between *HLA-DQB1* and rosacea, implying a possible correlation between rosacea and inflammatory or metabolic factors, providing hints for future studies in the mechanism of rosacea.

KEY MESSAGES

- *HLA-DQB1*03:03* allele, *HLA-DQB1*04:02* allele and *HLA-DQB1*03:03/05:02* genotype were significantly associated with rosacea.
- *HLA-DQB1*03:03* allele, *HLA-DQB1*05:01* allele and *HLA-DQB1*03:03/06:01* genotype were positively associated with rosacea severity.
- Neutrophils and HDL have mediation effects on the relationship between *HLA-DQB1*03:03* and risk or severity of rosacea.

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KEYWORDS

Rosacea; *HLA*; neutrophil; high-density lipoprotein

Introduction

Rosacea is a chronic inflammatory disease that primarily affects facial skin, which has a negative effect on patients' quality of life and mental health [1]. The major features of rosacea include erythema, telangiectasia, phymatous changes, ocular manifestations, and irritative sensations [2–4]. The prevalence of rosacea in China is approximately 3.48% based on a cross-section study [5].

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Supplemental data for this article can be accessed here.

More than one-third of rosacea patients have family history inclination [6–9]. Transcriptome profile analysis discovered that different subtypes of rosacea have certain genetic profiles [10,11]. Genome-wide association studies (GWAS) revealed some human leukocyte antigen (*HLA*) alleles were associated with the risk of rosacea among the Europeans. For example, *HLA-DMA/B* was correlated with immuno-inflammation phenotypes in rosacea, which was consistent with the inflammatory pathogenesis of rosacea [12,13]. However, the genetic background of rosacea has been lacking in Asian populations.

Besides, immune dysfunction is one of the dominating mechanisms of rosacea. Neutrophils, making up the majority of innate immune effector cells, react immediately against acute inflammation and immune reactions when exposed to pathogens [14]. Vast research confirmed that rosacea lesions are infiltrated with abundant neutrophils, suggesting a vital role of neutrophils played in the immune-related mechanism of rosacea. *HLA* is essential to immune reactions by presenting antigens and recruiting immunocytes including neutrophils. Besides, *HLA,* which presents antigens from extracellular sources, may explain the connection between microbes and rosacea and could participate in immune reactions of rosacea [13]. Hence, we speculate that *HLA* might mediate immune dysfunction in rosacea through mediating the expression of inflammatory cells, especially neutrophils. Therefore, this study aimed to investigate the mediation effect of neutrophils on the association between *HLA* and rosacea, if any.

Interestingly, rosacea is closely related to metabolic factors such as hypertension and dyslipidemia [15–17]. As reported, blood lipids, including total cholesterol, low-density lipoprotein and triglyceride, in rosacea patients were much higher than in healthy controls and obese patients were more susceptible to rosacea [17–20]. High-density lipoprotein (HDL) and low-density lipoprotein (LDL) are the main cholesterol-carrying lipoproteins [21]. Low level of HDL increases the risk of cardiovascular disease (CVD), since HDL has anti-inflammatory effects [22]. In contrast, down-regulation of LDL by medicines prevents atherosclerotic inflammation and lowers the incidence of CVD [23–27]. Interestingly, *HLA* alleles relate to HDL or LDL in patients with cardiovascular disease, atherogenesis, and rheumatoid arthritis. For instance, *HLA-DR* expression regulates LDL expression and immune-inflammatory cell content. Besides, genetic variants of *HLA-DQB1* connected with human longevity were associated with LDL/HDL ratio in a long-lived population [21,28–31]. Accordingly, we wonder if *HLA* alleles affect rosacea through mediating the expression of HDL and LDL.

In this study, we aimed at exploring the *HLA* typing among Chinese rosacea patients and investigating the mediation effect of inflammatory (neutrophils in peripheral blood) and metabolic (HDL, LDL) factors on the association between the susceptible *HLA* alleles and rosacea.

Materials and methods

Study design and population

This study was conducted from November 2017 to November 2019, in Xiangya Hospital of Central South University, Changsha. A total of 249 rosacea patients were recruited from the department of dermatology, diagnosed and divided into phenotypes of erythematotelangiectatic (ETR), papulopustular (PPR), and phymatous rosacea (PhR) by the 2004 diagnostic criteria determined by the National Rosacea Society Expert Committee [32]. 100 healthy controls, without a history of medication within the past three months, dieting, pregnancy, lactation, rosacea or other diseases, were recruited from the physical examination center. Patients with a history of medication within the past three months, dieting, pregnancy, or lactation, systemic diseases, specifically, cardiovascular disease, severe infections, mental illness, or other skin diseases (e.g. psoriasis and atopic dermatitis) that may interfere with the assessment of rosacea were excluded. The disease severity of rosacea was determined by the international investigator global rosacea severity scores (IGA scores) [33]. All assessments were completed by two dermatologists independently. Laboratory tests were conducted in Xiangya Hospital, including white blood cell count analysed by flow cytometer and serum HDL/LDL detected by colorimetry.

All participants signed written informed consents. This research was approved by the ethics review board of Xiangya Hospital Central South University and the approval number is NO.201611608, which covers the Declaration of Helsinki requirements.

DNA extraction and quality control

Peripheral venous blood was drawn from participants after 12 h overnight fasting. Centrifuging blood samples at 4000 g for 30 min to separate haemocytes and plasma. Genomic DNA was extracted from human blood cell samples by HiPure Blood & Tissue DNA Kit

(Magen, Cat#D3018-03). 1% of agarose gels were applied to test the degradation of DNA. The quality control of DNA was monitored by the NanoDrop spectrophotometer (ND-2000, Thermo Fisher Scientific).

HLA genotyping and imputation

Alleles from five *HLA* genes (*HLA-A, HLA-B, HLA-C, HLA-DQB1, HLA-DRB1*) were genotyped based on polymerase chain reaction with sequence-based typing (PCR-SBT) by TBG *HLAssure* SE DQB1 Locus SBT Kit (TBG Biotechnology Xiamen Inc). *HLA* allele results were analysed by supporting software AccuType (BioSoft, Oklahoma, USA).

Statistical analysis

The distribution of gender between patients and controls was compared by Chi-squared test, and other base characteristics by independent-sample t-test. When analyzing the relationship between the alleles or genotypes and rosacea, we took the highest frequent alleles or genotypes as the reference and calculated the odds ratios (OR) and 95% confidence intervals (CI) through logistic regression. The association between the alleles or genotypes and rosacea disease severity (IGA score) was analysed by linear regression. When the total associations (c) between the allele/genotype (independent X) and rosacea, rosacea severity was significant, the mediation analysis would be considered. The potential mediators (M) were neutrophils, HDL and LDL. Then the association (a) between X and each of the mediators was estimated, as well as the association between each of the mediators and Y after controlling for X (b) and the direct association (c′) between X and Y after controlling for M. When a and b were both significant associations, the indirect association (a × b) was calculated as the mediation effect. The percentage of the mediation effects equals to (a × b)/(a × b + c′). The pathways were all tested using bias-corrected bootstrapped 95% confidence intervals ($b = 5000$) and would be considered significant when a bootstrapped confidence interval does not include zero. All analyses were performed using SPSS and were considered significant when $p < 0.05$.

Results

HLA allele, genotype and risk of rosacea

Alleles of five *HLA* genes (*HLA-A, HLA-B, HLA-C, HLA-DRB, HLA-DQB1*) were genotyped among 100 rosacea patients (31.90 ± 10.53 years old) and 100 health controls (35.27 ± 8.85 years old), among which *HLA-A*11:01* (7.4% in rosacea, 1.6% in controls), *HLA-B*40:01* (8% in rosacea, 11.5% in controls), *HLA-C*01:02* (12% in rosacea, 10% in controls), *HLA-DRB*09:01* (10% in rosacea, 8.3% in controls), *HLA-DQB1*03:01* (7.8% in rosacea, 11.3% in controls) acquired the highest frequency in each gene. *HLA-A*, *HLA-B* and *HLA- DRB* were not relevant to rosacea. *HLA-C*03:04* allele was negatively associated with risk of rosacea (4.8% in rosacea, and 8.5% in controls; OR = 0.47, CI: 0.23 ∼ 0.94, $p = 0.033$). Contrarily, *HLA-DQB1*03:03* (10.8% in rosacea, and 8.5% in controls; OR = 1.84, CI: 0.97 ∼ 3.49, $p = 0.063$) and *HLA-DQB1*06:02* (3.5% in rosacea, and 2.3% in controls; OR = 2.26, CI: 0.87 ∼ 5.86, $p = 0.094$) alleles were positively associated with rosacea with marginal significance (Supplementary Table 1). Besides, *HLA-DQB1 03:03/03:03* (3.0% in cases, and 0.5% in controls; OR = 12.00, 95%CI: 1.12 ∼ 128.84, $p = 0.040$) genotypes were positively associated with risk of rosacea, and *HLA-DQB1 03:03/05:02* (3.5% in cases, and 1.5% in controls; OR = 4.67, 95%CI: 0.83 ∼ 26.24, $p = 0.080$) was found on the brink of significance correlated with rosacea (Supplementary Table 2). However, genotypes of other *HLA* genes had no significant associations with the risk of rosacea. Hence, *HLA-DQB1* may be a potential risk gene of rosacea.

HLA-DQB1 allele, genotype and risk, severity (IGA) of rosacea

To further explore the association between *HLA-DQB1* alleles and rosacea, we enlarged the sample size to 399 individuals, including 249 rosacea patients and 150 healthy controls. The demographic information of selected subjects was listed in Table 1 and there was no significant difference between case and control groups in the distribution of gender or age. The proportion of ETR, PPR, and PhR rosacea patients were 58.2%, 35.7%, and 6.0%, respectively (Table 1).

Twelve alleles and 9 genotypes were detected on *HLA-DQB1* among 399 individuals, of which the

Table 1. The demographic and clinical features of the rosacea cases and controls.

Characteristics		Rosacea ($n = 249$)	Controls ($n = 150$)	*p-value*
Sex	Male	18 (7.2%)	14 (9.3%)	0.453*
	Female	231 (92.8%)	136 (90.7%)	
Age (year, mean ± SD)		33.02 ± 10.89	34.21 ± 8.83	0.234#
Phenotype (N, %)	ETR	145, 58.2%	–	–
	PPR	89, 35.7%		
	PhR	15, 6.0%		

*Chi-square test; #t-test.

Table 2. Association between *HLA-DQB1* allele, genotype and rosacea *.

Variable	Rosacea freq	Control freq	OR (95%CI)	*p-value*
Allele ($2N = 798$)				
*DQB1*03:01*	0.130	0.074	Ref.	
*DQB1*02:01*	0.035	0.016	1.22 (0.59, 2.560)	0.591
*DQB1*02:02*	0.016	0.014	0.66 (0.28, 1.56)	0.342
*DQB1*03:02*	0.038	0.025	0.79 (0.41, 1.53)	0.484
*DQB1*03:03*	0.128	0.070	41.89 (9.80, 179.09)	**$4.7^{*}10^{-7}$**
*DQB1*04:01*	0.019	0.018	0.60 (0.27, 1.34)	0.215
*DQB1*04:02*	0.003	0.009	0.16 (0.03, 0.81)	**0.026**
*DQB1*05:01*	0.020	0.006	1.84 (0.64, 5.30)	0.257
*DQB1*05:02*	0.071	0.040	1.06 (0.61, 1.86)	0.827
*DQB1*05:03*	0.010	0.028	1.60 (0.67, 3.83)	0.292
*DQB1*06:01*	0.103	0.054	1.17 (0.70, 1.97)	0.555
*DQB1*06:02*	0.036	0.021	0.91 (0.46, 1.82)	0.799
Genotype ($N = 399$)				
*DQB1*03:01/03:01*	0.018	0.023	Ref.	
*DQB1*03:01/03:03*	0.033	0.035	1.19 (0.34, 4.14)	0.780
*DQB1*03:01/05:02*	0.033	0.013	3.34 (0.80, 13.94)	0.098
*DQB1*03:01/06:01*	0.028	0.020	1.77 (0.46, 6.78)	0.406
*DQB1*03:02/03:03*	0.020	0.010	2.57 (0.54, 12.17)	0.234
*DQB1*03:03/03:03*	0.028	0.008	4.71 (0.94, 23.68)	0.060
*DQB1*03:03/05:02*	0.033	0.008	5.57 (1.13, 27.52)	**0.035**
*DQB1*03:03/06:01*	0.055	0.028	2.57 (0.76, 8.75)	0.131
*DQB1*05:02/06:01*	0.023	0.013	2.31 (0.53, 10.10)	0.264

*Allele or genotype frequency less than 0.030 were not presented.
p-value in bold indicates statistical significance.

Table 3. Association between *HLA-DQB1* allele, genotype and rosacea severity.

Variable	b	SE	*p*-value
Allele ($2N = 798$)			
*DQB1*03:01*	Ref.		
*DQB1*02:01*	0.142	0.252	0.575
*DQB1*02:02*	−0.385	0.316	0.225
*DQB1*03:02*	0.128	0.243	0.599
*DQB1*03:03*	1.434	0.217	**$2.0^{*}10^{-10}$**
*DQB1*04:01*	−0.437	0.289	0.133
*DQB1*04:02*	−0.855	0.492	0.084
*DQB1*05:01*	0.894	0.33520	**0.008**
*DQB1*05:02*	0.043	0.19842	0.828
*DQB1*05:03*	0.397	0.28722	0.169
*DQB1*06:01*	0.177	0.18418	0.337
*DQB1*06:02*	0.202	0.24939	0.419
Genotype ($N = 399$)			
*DQB1*03:01/03:01*	Ref.		
*DQB1*03:01/03:03*	−0.063	0.415	0.881
*DQB1*03:01/05:02*	0.549	0.459	0.241
*DQB1*03:01/06:01*	0.043	0.421	.920
*DQB1*03:02/03:03*	0.104	0.511	.840
*DQB1*03:03/03:03*	0.295	0.449	.517
*DQB1*03:03/05:02*	0.875	0.499	.090
*DQB1*03:03/06:01*	0.998	0.472	**.040**
*DQB1*05:02/06:01*	0.438	0.501	.390

p in bold indicates statistical significance.

frequency of *HLA-DQB1*03:01* was the highest (20.4% in controls). The *HLA-DQB1*03:03* allele was positively associated with risk of rosacea (12.8% in cases, and 7.0% in controls; OR = 41.89, 95% CI: 9.80 ~ 179.09, $p = 4.7^{*}10^{-7}$), while *HLA-DQB1*04:02* was negatively associated with risk of rosacea (0.3% in cases, and 0.9% in controls; OR = 0.16, 95% CI: 0.03 ~ 0.81, $p = 0.026$). Besides, the *HLA-DQB1* genotype *03:03/05:02* was positively associated with risk of rosacea (3.3% in cases, and 0.8% in controls; OR = 5.57, 95% CI: 1.13 ~ 27.52, $p = 0.0351$) after enlarging sample size. Other alleles or genotypes had no significant associations with the risk of rosacea (Table 2).

Except for risk of rosacea, alleles and genotypes of *HLA-DQB1* were strongly associated with disease severity of rosacea (Table 3). *HLA-DQB1*03:03* and *HLA-DQB1*05:01* were positively correlated with disease severity (b = 1.434, $p = 2.0^{*}10^{-10}$; b = 0.894, $p = 0.008$, respectively). In addition, *HLA-DQB1*03:03/06:01* genotype had a positive correlation with IGA scores (b = 0.998, $p = 0.040$).

Mediation role of neutrophils, HDL and LDL on the relationship between HLA-DQB1 allele/genotype and rosacea risk or rosacea severity

Since *HLA-DQB1* might be a potential risk gene, and *HLA* locus could regulate neutrophils, HDL and LDL, we herein collected the laboratory tests of all participants to explore the association between *HLA-DQB1* allele/genotype, neutrophils, HDL, LDL, rosacea risk, and rosacea severity (Table 4, Figure 1, Supplementary Table 3).

In the associations between *HLA-DQB1* and the potential mediators for rosacea risk, *HLA-DQB1*03:03* was positively associated with neutrophils ($\beta = 0.564$, 95% CI: 0.213 ~ 0.913), while negatively correlated with HDL ($\beta = -0.172$, 95% CI: −0.273~−0.070). As for rosacea severity, *HLA-DQB1*03:03* was positively associated with neutrophils ($\beta = 0.565$, 95% CI: 0.217 ~ 0.903), while negatively correlated with HDL ($\beta = -0.172$, 95% CI: −0.278~−0.070). As for the association between potential mediators and rosacea risk, or severity, neutrophils were positively associated with rosacea risk ($\beta = 0.839$, 95% CI: 0.476 ~ 1.338) and disease severity ($\beta = 0.329$, 95% CI: 0.196 ~ 0.458), while HDL was negatively associated with rosacea ($\beta = -2.706$, 95% CI: −3.971~−1.729) and IGA score ($\beta = -1.154$, 95% CI: −1.649~−0.624).

Furthermore, we analysed the mediation effects of neutrophils and HDL on the relationship between the *HLA-DQB1* allele and rosacea risk, or severity (Table 5). The mediation effect of neutrophils on the relationship between *HLA-DQB1*03:03* and rosacea risk was 0.47 (95% CI: 0.16 ~ 0.92), and the mediation ratio was 10.9%. The mediation effect of neutrophils on the relationship between *HLA-DQB1*03:03* and rosacea severity was 0.18 (95% CI: 0.06 ~ 0.34, mediation

Table 4. Association between *HLA-DQB1* gene, neutrophils, HDL, LDL, rosacea and rosacea severity (IGA).

Y	X	M	c path β (95% CI)[a]	a path β (95% CI)[a]	b path β (95% CI)[a]
Rosacea	*DQB1*03:03*	Neutrophils	**3.850 (2.729, 18.037)**	**0.564 (0.213, 0.913)**	**0.839 (0.476, 1.338)**
Rosacea	*DQB1*03:03*	HDL	**3.664 (2.183, 5.145)**	**−0.172 (−0.273, −0.070)**	**−2.706 (−3.971, −1.729)**
Rosacea	*DQB1*03:03*	LDL	**3.744 (2.703, 17.946)**	0.074 (-0.110, 0.251)	**0.482 (0.037, 1.011)**
IGA	*DQB1*03:03*	Neutrophils	**1.249 (0.977, 1.516)**	**0.565 (0.217, 0.903)**	**0.329 (0.196, 0.458)**
IGA	*DQB1*03:03*	HDL	**1.235 (0.936, 1.517)**	**−0.172 (−0.278, −0.070)**	**−1.154 (−1.649, −0.624)**
IGA	*DQB1*03:03*	LDL	**1.414 (1.136, 1.676)**	0.074 (−0.111, 0.255)	0.264 (−0.041, 0.555)
Rosacea	*DQB1*03:03/05:02*	Neutrophils	1.395 (−0.380, 16.036)	1.106 (−0.098, 2.220)	0.500 (−0.032, 1.583)
Rosacea	*DQB1*03:03/05:02*	HDL	**1.695 (0.139, 16.381)**	(−0.219 (−0.473, 0.036)	−0.107 (−3.686, 3.377)
Rosacea	*DQB1*03:03/05:02*	LDL	**1.723 (0.260, 16.452)**	0.123 (−0.293, 0.615)	−0.043 (−2.653, 2.331)
IGA	*DQB1*03:03/06:01*	Neutrophils	0.689 (−0.138, 1.601)	0.942 (−0.082, 1.838)	**0.328 (0.076, 0.510)**
IGA	*DQB1*03:03/06:01*	HDL	0.788 (−0.152, 1.694)	−0.184 (−0.365, 0.007)	−1.145 (−2.606, 0.392)
IGA	*DQB1*03:03/06:01*	LDL	0.835 (−0.112, 1.736)	**0.361 (0.004, 0.719)**	0.451 (−0.157, 1.131)

[a]bias-corrected bootstrapped 95% confidence interval.
β in bold indicates statistical significance.
Threshold value: Neutrophils: 3.5–9.5 * 109/L; HDL: 1.04–1.55 * mmol/L; LDL: 1.55–3.19 * mmol/L.

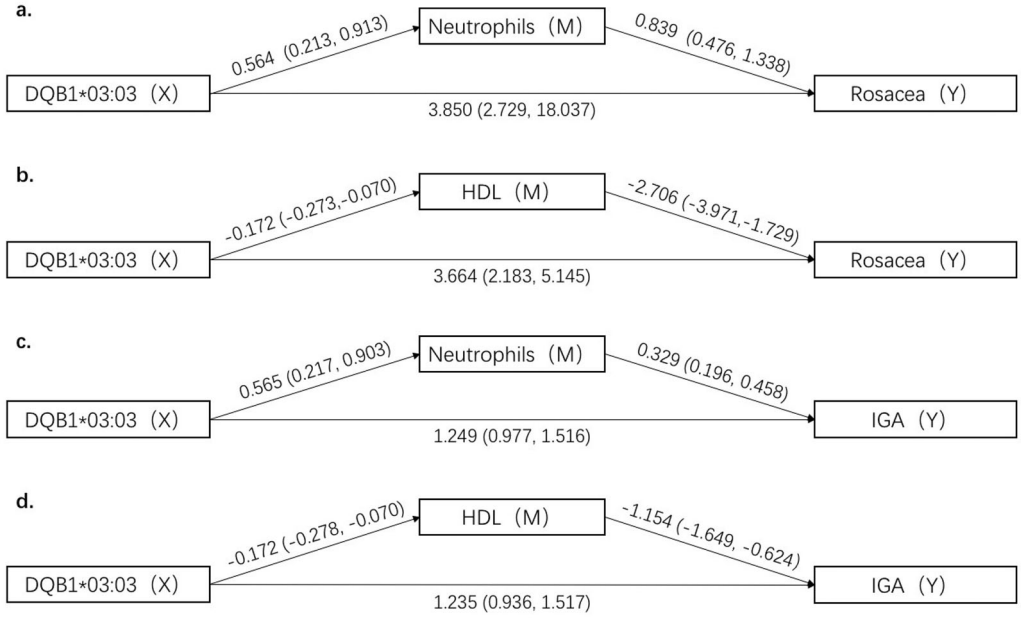

Figure 1. Mediation analysis for *HLA-DQB1**03:03, neutrophils, HDL and rosacea risk or severity. HDL: high-density lipoprotein; IGA: disease severity of rosacea.

Table 5. Mediation of neutrophils and HDL, LDL on the relationship between *HLA-DQB1* allele and rosacea or rosacea severity.

Dependent	Mediator	Independent	Direct association β (95% CI)[a]	Indirect association β (95% CI)[a]	Percent of mediation	Percent of direct association
Allele						
*DQB1*03:03 (2N = 206)*	Neutrophils	rosacea	3.84 (2.72, 18.04)[b]	0.47 (0.16, 0.92)[b]	10.9	89.1
	HDL	rosacea	3.66 (2.52, 17.89)[b]	0.46 (0.18, 0.86)[b]	11.17	88.83
	Neutrophils	IGA score	1.25 (0.98, 1.52)	0.18 (0.06, 0.34)	12.59	87.41
	HDL	IGA score	1.24 (0.94, 1.52)	0.20 (0.07, 0.37)	13.89	86.11

[a]bias-corrected bootstrapped 95% confidence interval.
[b]based on logistic regression analysis and the results are expressed in a log-odds metric.
IGA: disease severity of rosacea.

ratio = 12.59%). For HDL, the mediation effect was 0.46 (95% CI: 0.18 ~ 0.86) on the relationship of *HLA-DQB1*03:03* and rosacea risk (mediation ratio = 11.17%), 0.20 (95% CI: 0.07 ~ 0.37) on the relationship of *HLA-DQB1*03:03* and rosacea severity (mediation ratio = 13.89%).

Discussion

In this study, we identified new susceptible *HLA* alleles of rosacea, especially *HLA-DQB1*03:03*, among Chinese population, and clarified the mediation effect of neutrophils and HDL in the association between *HLA-DQB1*03:03* and rosacea.

Genetic background was considered important in the incidence and severity of rosacea by twin studies [10]. GWAS in Europeans revealed an association between *HLA* alleles and rosacea risk/severity, including *HLA-DRB1*03:01*, *HLA-DQB1*02:01*, and *HLA-DQA1*05:01* [13]. However, relevant research in Asia has been absent. We identified *HLA-C*03:04* and *HLA-DQB1*04:02* as protective alleles, while *HLA-DQB1*03:03* allele and *HLA-DQB1*03:03/05:02* genotype as risk factors. Besides, *HLA-DQB1*03:03*, *HLA-DQB1*05:01*, and *HLA-DQB1*03:03/06:01* were positively associated with rosacea severity. The discrepancy between our study and the previous studies may attribute to the differences in ethnicity, or the relatively limited sample size. Interestingly, *HLA-DQB1*03:03*, which was associated with both risk and severity of rosacea in our study, was reported to have linkage with various immune diseases, including pemphigus vulgaris, pemphigus foliaceous, multiple sclerosis and auto-immune thyroid disease [34–36]. Hence, we speculated that *HLA-DQB1*03:03* was involved in the mechanism of rosacea through inducing immunologic disorder. In brief, our study further confirmed the concept of an inflammatory genetic component in rosacea.

Lesions of rosacea are infiltrated by inflammatory cells, especially neutrophils, which was confirmed by immunohistochemistry and transcriptome analysis [37,38]. Moreover, topical medications like metronidazole alleviate disease condition by inhibiting neutrophils [39–41]. However, obtaining neutrophils from the lesions is invasive and the fluctuation of neutrophil levels in peripheral blood reflects the changes in the individual's overall inflammatory status to some extent. Hence, we explored the mediation effect of neutrophils in peripheral blood on the relationship between *HLA-DQB1*03:03* and rosacea risk or severity, revealing the mediation effect at 10.9% and 12.59%, respectively. This indicates that neutrophils play a role in the *HLA*-related immune mechanism in rosacea. Since neutrophils can be stimulated and activated by MHC class II alleles [42–46], we presume that *HLA-DQB1*03:03* might recruit and stimulate neutrophils in rosacea. Still, the underlying mechanism needs further exploration.

Rosacea has been reported to be highly related to metabolic factors, such as dyslipidemia [15–17], indicating that metabolic pathway might get involved in rosacea. *HLA-DQB1* alleles, proved to be associated with risk and severity of rosacea in our study, have been found related to abnormal HDL-c levels in a cross-sectional study among the Chinese long-living population [21]. Consequently, we explored the mediating effects of HDL and LDL in *HLA-DQB1*-influenced risk and severity of rosacea. HDL expression was found negatively correlated with *HLA-DQB1*03:03* and rosacea risk or severity, indicating a protective role of HDL in rosacea, with the mediation effect on the relationship between *HLA-DQB1*03:03* and rosacea risk/severity as 11.17% and 13.89%, respectively. The association between rosacea and abnormal lipid metabolism provides a novel hint for the mechanism of rosacea. HDL levels are negatively associated with gene expression of human cathelicidin (LL-37), a key peptide involved in the pathogenesis of rosacea, which explained the possible relevance between HDL and rosacea [19,47]. In addition, human serum paraoxonase (PON1) with low activity enables HDL to be more susceptible to oxidation, which may increase the risk of rosacea [48–50]. Conclusively, *HLA-DQB1* alleles might induce rosacea through inhibiting HDL expression, which needs further exploration.

The strengths of our study were the *HLA* typing in Asian rosacea populations and the mediation analysis of both inflammatory and metabolic factors, which fulfil the blank of the genetic research of Asian groups and provide new hints for the mechanism of rosacea. The limitation of our research is the insufficient sample sizes. Though we recruited nearly four hundred people, the positive number of each *HLA* allele was still limited.

In conclusion, novel *HLA* alleles are associated with rosacea risk and severity in the Chinese group. *HLA-DQB1*03:03* is an important genetic predisposition for rosacea. Besides, we explored the mediation effect of both neutrophils and HDL on the relationship between *HLA-DQB1* alleles and rosacea risk or severity, implicating that inflammatory and metabolic-related factors might get involved in the mechanism of rosacea under the genetic basis. This discovery provides new directions for targeted therapy and comprehension of rosacea.

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Author contributions

Conception and design: J.L. and Y.T. Financial support: J.L. and H.X. Collection and assembly of data: W.X, Q.Z., T.L., and Z.D. Data analysis and interpretation: X.H. and Y.T. Manuscript writing and revision: W.X. Final approval of manuscript: J.L. and Y.T.

Disclosure statement

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Data availability statement

Data are contained within this article or its supplementary material.

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