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Association of *Dectin-1* and *DC-SIGN* gene single nucleotide polymorphisms with fungal keratitis in the northern Han Chinese population

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Purpose: Dendritic cell-associated C-type lectin-1 (Dectin-1) and dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) play a crucial role in the early procedure of fungal pathogen defenses. The present study evaluated the associations between *Dectin-1* and *DC-SIGN* gene single nucleotide polymorphisms (SNPs) and susceptibility to fungal keratitis (FK) in the northern Han Chinese population.
Methods: The polymorphisms of *Dectin-1* (rs17206002, rs3901533, rs11053613, and rs3901532) and *DC-SIGN* (rs4804803, rs2287886, rs735239, and rs735240) for 109 FK patients and 220 matched healthy controls were determined by PCR and DNA direct sequencing assay.
Results: Each SNP was consistent with Hardy–Weinberg equilibrium ($p>0.05$). The frequencies of genotypes and alleles for rs735239 and rs735240 (*DC-SIGN*) showed statistical differences between patients and control groups ($p<0.05$). The wild G allele of rs735239 and the wild A allele of rs735240 were significantly higher in patients ($p = 0.003$, OR = 1.766, 95% confidence interval [CI] 1.207–2.585; $p = 0.014$, OR = 1.609, 95% CI 1.100–2.355, respectively). No association with a risk of FK was found for the remaining SNPs ($p>0.05$) even after ruling out clinical characteristics, such as severity degree and case history. Carriers of the haplotype TC (rs4804803 and rs2287886) had a higher risk of developing fungal keratitis ($p = 0.007$, OR = 1.710, 95% CI 1.154–2.534). The distribution of haplotypes AG and GA (rs735239 and rs735240) between the two groups also showed significant differences ($p = 0.014$, $p = 0.003$, respectively).
Conclusions: Two SNPs of *DC-SIGN* (rs735239 and rs735240) are associated with susceptibility to FK in the northern Han Chinese population. The haplotypes of *DC-SIGN* may be susceptible to the risk of FK, whereas the analysis of *Dectin-1* gene polymorphisms showed no significant association with FK risk. Further research with a larger sample is recommended.

Fungal keratitis (FK) is recognized as one of most serious vision-threatening corneal infectious diseases because it can result in endophthalmitis, corneal perforation, and vision loss. Although new therapies, such as collagen cross-linking [1], voriconazole [2], and therapeutic keratoplasty [3], have emerged in clinical practice, their efficacies are still below expectations. Because of the penetrating ability of the fungal hyphae, pathogens can spread widely and deeply in the early stages, and graft re-infection may occur after corneal transplantation [4-6]. FK remains a therapeutic challenge to ophthalmologists because of the lack of established gold standard treatments and guidelines and because the incidence and etiology of FK are different among particular geographic areas. Due to many factors, such as the wide use of broad-spectrum antibiotics, immunosuppressants, and contact lens, FK has been reported more frequently in recent years [4,5], with *Fusarium*, *Aspergillus*, and *Candida albicans* being the main pathogens [4-9]. Therefore, effective preventive targets are necessary.

Innate immunity recognizes pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), which could mediate the inflammatory response and play a pivotal role in anti-infection defense [10-12]. Early recognition of *Aspergillus fumigatus* is crucial for downstream immune response and conidia clearance [12-14]. C-type lectin receptors (CLRs) are members of PRRs and are important in the response to fungal infection. CLRs, such as *Dectin-1* and *DC-SIGN*, are considered as pivotal cell membrane PRRs in the innate immune system against fungal infection [14-16]. Studies have shown that nonimmune cells, such as human epidermal cells and corneal epithelium cells, can be involved in the antifungal immune response via inherent PRRs [10,11,13,17,18].

Genetic polymorphisms are recognized as potential factors that could affect human susceptibility and induce

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some infectious diseases, and some infectious diseases are correlated with environmental factors and gene polymorphisms [19,20]. Through genetic polymorphism studies, researchers have revealed the essence of individual differences in biologic function. Several studies have confirmed that *Dectin-1* and *DC-SIGN* gene variants could result in altered cytokine release in fungal infection response [18-21], but little work has been carried out on the association between gene polymorphisms and FK. In this study, we investigated the distribution of *Dectin-1* and *DC-SIGN* SNPs in the northern Han Chinese population and explored the relationships between these SNPs and susceptibility of FK.

METHODS

Study population and classification: The study group consisted of 109 FK patients recruited from the Ophthalmology Department of Qingdao University Affiliated Hospital in Qingdao of China, from January 2008 to December 2012 (men 75; mean age: 54.61 ± 10.72 years). The control group comprised 220 unrelated healthy individuals (118 males; mean age 54.61±11.67 years) matched on gender and age from this hospital in the same period.

The diagnosis of FK was based on laboratory examinations (fungal culture) and clinical manifestation [22]. Among these patients, 68 cases had a history of corneal trauma. Patients were divided into three subgroups according to clinical features: mild (13 cases), defined as the area of corneal ulcer is smaller than 3 mm^2 or the depth is less than one-third of the corneal thickness, a partial corneal edema, without an anterior chamber abscess; moderate (29 cases), defined as the area of corneal ulcer is 3–6 mm^2 or the depth is between one-third and two-thirds of the corneal thickness, larger corneal edema, anterior chamber abscess; and severe (67 cases), defined as the corneal ulcer area is more than 6 mm^2 or the depth is larger than two-thirds of the corneal thickness, cloudy corneal edema, folded Descemet's membrane, exudation and empyema in anterior chamber, significant vision loss, or unsuccessful drug treatment.

All the subjects were northern Han Chinese residents and had no relationship with each other. People with significant illness, such as type 2 diabetes mellitus, heart disease, cancer, hypertension, and atherosclerosis, were excluded from the study. There was no statistical difference in age and gender between the two groups ($p>0.05$). Informed consent of all participates was obtained, and the protocol of this study was approved by the Institutional Ethics Committee of Qingdao University Affiliated Hospital. All DNA samples and data in this study were handled anonymously.

Selection of SNPs and genotyping: Peripheral venous blood (2 ml) was collected in EDTA tube from all subjects. Genomic DNA was extracted using the genomic DNA purification kit (DP319, Tiangen Biotech CO., Beijing, China) following the manufacturer's instruction. All genomic DNA was stored at -80 °C freezer until analysis. All single nucleotide polymorphisms (SNPs) were selected based on previous literature and information, including the NCBI GenBank, dbSNP, and HapMap databases. The selected SNPs were in 5' noncoding region of *Dectin-1* and the promoter region of *DC-SIGN*, which may influence gene transcription. Primers were designed with Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA) and synthesized by Sangon Biology company (Shanghai, China). PCR (Table 1) was performed in the Eppendorf PRO PCR system (Hamburg, German), using a PCR amplification kit (code No. RR003A, TaKaRa, Japan). All SNPs were genotyped by ABI 3730 XL system (Marjorbio Engineering Limited Company, Shanghai, China). DNA sequences were read by Chromas 2.3 software (Technelysium Pty Ltd., Tewantin, Australia). About 5% of samples were randomly selected and retested for internal quality control.

Table 1. Primer sequences for each SNP and PCR conditions.

Gene	Gene location	SNPs		Primer pairs	Product length	Anneling temperature (°C)	Extendding time(s)
Dectin-1	Intron	rs17206002 rs3901533 rs11053613 rs3901532	F	TGACTACAATAATCAGGACACTACC	777 bp	64	47
			R	TAGCCATTGTCTTCTCCTCCA			
DC-SIGN	Promoter	rs4804803 rs2287886	F	ATTATGATTCTGCCCCAACTC	424 bp	63	27
			R	CAGCTTTTATTTCCCACCCT			
DC-SIGN	Promoter	rs735239 rs735240	F	TAGATGGTGGGGCTGAGACT	407 bp	62	25
			R	GCATACAGAAACCCCGTTGT			

Statistics: Each SNP in the controls was tested for Hardy–Weinberg equilibrium (HWE) by Haploview 4.2 (Massachusetts Institute of Technology, Cambridge, MA), and a value of $p<0.05$ was considered a deviation from HWE. Statistical comparison was performed by independent Student *t* test or one-way analysis of variance. Pearson's χ^2 or Bonferroni-corrected multiple comparison tests were used to identify differences in allele and genotype frequencies of the various SNPs and haplotypes between the patients and healthy controls. The odds ratios (ORs), 95% confidence intervals (CIs), and two-tailed p values were calculated for all tests. Statistical analysis was performed by SPSS 17.0 software (SPSS Inc., Chicago, IL). The level of significance for all statistical tests was defined as $p<0.05$.

RESULTS

HWE and case-control genotype analyses: All SNPs of the healthy controls met HWE ($p>0.05$); the minor allele frequencies of all SNPs in this group were over 5%. The genotypes and allele frequencies are shown in Table 2. Differences in the genotypes and allele distributions of SNPs rs735239 and rs735240 (*DC-SIGN*) between the two groups were statistically significant ($p<0.05$). A significant under-representation of the rs735239 AA homozygote was observed in patients when compared to healthy controls ($p = 0.019$), whereas the rs735240 AA homozygote was significantly higher among patients ($p = 0.033$). The wild G allele of rs735239 and the wild A allele of rs735240 were significantly higher in FK patients ($p = 0.003$, OR = 1.766, 95% CI = 1.207–2.585; $p = 0.014$, OR = 1.609, 95% CI = 1.100–2.355, respectively). Other SNPs did not differ between the two groups.

Associations with severity classification and corneal trauma history: We divided the subjects into three groups based on disease severity and compared the genotype frequencies with controls (Table 3). The allele and genotype distributions of all SNPs in *Dectin-1* and *DC-SIGN* did not show any trend of association with the severity of keratitis ($p>0.05$).

We divided the cases into two groups according to whether or not the individual had a history of corneal trauma and compared this with the healthy controls (Table 4). No significant association was observed in the SNPs within *Dectin-1* and *DC-SIGN* ($p>0.05$).

Linkage disequilibrium and haplotype analyses: Linkage disequilibrium (LD) and haplotype analyses were performed with Haploview 4.2 to identify the genetic relationship between each SNP on *Dectin-1* and *DC-SIGN* (Table 5 and Table 6). The haplotype analysis of the *DC-SIGN* gene region, which was divided into two LD blocks (block 1 included rs2287886 and rs4804803; block 2 included rs735239 and rs735240), revealed a strong association with FK and an extremely high magnitude of LD between the SNPs (pair-wise D' statistic >0.95) (Figure 1). Comparing the three haplotypes of *DC-SIGN* block 1, we found that in two groups Haplotype CT, which included a minor allele of rs2287886 and a major allele of rs4804803, was statistically significant (OR = 1.710, 95% CI = 1.154–2.534, $p = 0.007$), suggesting that Haplotype CT (rs2287886–rs4804803) was correlated with an increased risk of FK. Moreover, in block 2, Haplotypes AG and Haplotypes GA (rs735239–rs735240) showed significant differences between the cases and control subjects ($p<0.05$). Interestingly, Haplotypes AG, which included major alleles of rs735239 and rs735240, showed a reduced risk of FK, while Haplotypes GA, which included minor alleles of rs735239 and rs735240, showed an increased risk (Haplotypes AG: OR = 0.621, 95% CI = 0.425–0.909, $p = 0.014$; Haplotypes GA: OR = 1.949, 95% CI = 1.242–3.059, $p = 0.003$). However, as shown in Table 6, in the haplotype analysis of *Dectin-1*, one LD block (rs17206002, rs3901533, rs11053613, rs3901532) showed no significant association with FK ($p>0.05$).

DISCUSSION

This is the first study to investigate the association between *Dectin-1* and *DC-SIGN* SNPs and FK in the northern Chinese Han population. The present study aimed to analyze the eight SNPs (rs17206002, rs3901533, rs11053613, rs3901532, rs4804803, rs2287886, rs735239 and rs735240) in FK patients and control subjects to identify relationships between them. Our results showed that two SNPs (rs735239 and rs735240) and three haplotypes (CT, AG, and GA) in *DC-SIGN* were significantly associated with FK, indicating that the *DC-SIGN* promoter polymorphisms contribute to the risk of FK.

The interaction between host PRRs and PAMPs of pathogenic microorganisms plays a key role in the innate immune response, which occurs at an early stage of fungal infection. PRRs can initiate the body's nonspecific immune response, recruit a large number of inflammatory cells, and trigger a specific immune response [12,14,23]. Dectin-1 and DC-SIGN are popular members of the CLR superfamily as Ca^{2+}-dependent type II transmembrane protein receptors, which are closely related to antifungal innate immunity. Dectin-1 and DC-SIGN can recognize the β-1, 3/1, 6-glucans in the cell membrane of fungi and then bind with endogenous ligands on the surface of T cells and endothelial cells [24-26]. Many studies have shown that Dectin-1 and DC-SIGN are involved in the identification of fungal pathogens and in the induction of antifungal Th1 and Th17 immune responses [27-29].

In our study, we hypothesized that mutations in the *Dectin-1* and *DC-SIGN* genes, which participate in immune

Table 2. Genotype and allele distribution of FK patients compared with controls.

SNP	Genotype/Allele	Cases (n=109) N(%)	Controls (n=220) N(%)	*P**	OR (95%CI) **
rs17206002	AA	87 (79.8)	184 (83.6)	0.392	0.774(0.430–1.394)
	TA	22 (20.2)	36 (16.4)		
	T	22 (10.1)	36(8.2)	0.416	0.794 (0.455–1.386)
	A	196 (89.9)	404 (91.8)		
rs11053613	AA	82 (75.2)	169 (76.8)	0.750	0.917(0.536–1.566)
	GA	27 (24.8)	51 (23.2)		
	G	27 (12.4)	51 (11.6)	0.767	0.927 (0.564–1.525)
	A	191(87.6)	389 (88.4)		
rs3901532	AA	2 (1.8)	6 (2.7)	0.882	
	GA	41 (37.6)	81 (36.8)		
	GG	66 (60.6)	133 (60.5)		
	A	45 (20.6)	93 (21.1)	0.883	0.977 (0.711–1.341)
	G	173 (79.4)	347 (78.9)		
rs3901533	GG	2 (1.8)	5 (2.3)	0.949	
	GT	42 (38.5)	82 (37.3)		
	TT	65 (59.6)	133 (60.5)		
	G	46 (21.1)	92 (20.9)	0.955	1.009 (0.737–1.382)
	T	172 (78.9)	348 (79.1)		
rs4804803	CC	1 (0.9)	5 (2.3)	0.407	
	CT	17 (15.6)	44 (20.0)		
	TT	91 (83.5)	171 (77.7)		
	C	19 (8.7)	54 (12.3)	0.171	0.710 (0.432–1.167)
	T	199 (91.3)	386 (87.7)		
rs2287886	CC	14 (12.8)	23 (10.5)	0.407	
	TC	46 (42.2)	81 (36.8)		
	TT	49 (45.0)	116 (52.7)		
	C	74 (33.9)	127 (28.9)	0.183	1.176 (0.929–1.489)
	T	144 (66.1)	313 (71.1)		
rs735239	AA	73 (67.5)	176 (80.0)	0.019	
	GA	30 (27.5)	40 (18.2)		
	GG	6 (5.5)	4(1.8)		
	G	42 (19.3)	48 (10.9)	0.003	1.766 (1.207–2.585)
	A	176 (80.7)	392 (89.1)		

SNP	Genotype/Allele	Cases (n=109) N(%)	Controls (n=220) N(%)	*P**	OR (95%CI) **
rs735240	AA	12 (11.0)	9 (4.1)	0.033	
	AG	36 (33.0)	66 (30.0)		
	GG	61 (56.0)	145 (65.9)		
	A	60 (27.5)	84 (19.1)	0.014	1.609 (1.100–2.355)
	G	158 (72.5)	356 (80.9)		

*: χ^2 test; **: OR=odds ratio, CI=confidence interval.

Table 3. Allele and genotype distribution by severity of FK.

SNP	Genotype /Allele	Cases (n=109) N (%)			Controls (n=220) N (%)	χ^2	*P*
		Mild	Moderate	Severe			
rs17206002	AA	8(61.5)	25 (86.2)	54 (80.6)	184 (83.6)	4.567	0.206
	TA	5 (38.5)	4 (13.8)	13 (19.4)	36 (16.4)		
rs11053613	AA	11 (84.6)	22 (75.9)	49 (73.1)	169 (76.8)	0.904	0.824
	GA	2 (15.4)	7 (24.1)	18 (26.9)	51 (23.2)		
rs3901532	AA	0 (0.0)	0 (0.0)	2 (3.0)	6 (2.7)	7.532	0.274
	GA	9 (69.2)	9 (31.0)	23 (34.3)	81 (36.8)		
	GG	4 (30.8)	20 (69.0)	42 (62.7)	133 (60.5)		
rs3901533	GG	0 (0.00)	0 (0.00)	2(3.0)	5 (2.3)	7.246	0. 299
	GT	9 (69.2)	9 (31.0)	24 (35.9)	82 (37.3)		
	TT	4 (30.8)	20(69.0)	41(61.1)	133(60.5)		
rs4804803	CC	0 (0.0)	1 (3.4)	0 (0.0)	5 (2.3)	4.408	0. 622
	CT	1 (7.7)	6 (20.7)	10 (14.9)	44 (20.0)		
	TT	12 (92.3)	22 (75.9)	57 (85.1)	171 (77.7)		
rs2287886	CC	1 (7.7)	5 (17.2)	8 (11.9)	23 (10.5)	4.740	0. 578
	TC	4 (30.8)	14 (48.3)	28 (41.8)	81 (36.8)		
	TT	8 (61.5)	10 (34.5)	31 (46.3)	116 (52.7)		

reactions, are likely to lead to changes of corresponding encoded products and affect the host's defense to fungal infection. It is well established that some elements in a gene's noncoding region could affect gene transcription and translation [21,30-32]. The promoter region plays an important role in the regulation of gene transcription, and the regulation of promoters on transcription is determined by plural regulatory sequences. Although a single nucleotide substitution may not generate a new transcription recognition sequence, it may change the ability of the transcription factor binding with the corresponding loci.

As mentioned above, rs3901532 and rs3901533 are in the introns of the *Dectin-1* gene. They are assumed to influence other neighboring loci to change the susceptibility of a fungal infection. However, we found no statistically significant difference in the relationship between *Dectin-1* and FK. Studies have shown that some SNPs of *Dectin-1* are associated with a host's susceptivity to fungal infections, such as *Aspergillus* and *Candida* [19,32,33]. Because *Dectin-1* mutations are rarely reported in the Chinese population, we suggest that our inconsistent result might stem from the differences between populations. Moreover, further study with a larger sample size is necessary. Dectin-1 activates immunologic effector cells via the c-rapidly accelerated fibrosarcoma proteins (Raf-1) or Myeloid differentiation factor 88 (MyD88)-dependent signaling pathways to produce a large number of cytokines and chemokines. *Dectin-1* receptors showed protective effects in the mouse lung *A. fumigatus* infection model [29]. Brown et al. [34] found that *Dectin-1* gene-deficient mice injected with *Candida albicans* recruited fewer inflammatory cells than wild-type mice, but the mortality of these mice with no *Dectin-1* gene increased. Sainz J et al. [19] reported that *Dectin-1* rs3901533T/T and rs7309123G/G genotype carriers had a significantly increased risk of invasive pulmonary *Aspergillus* infection (IPA) and a galactomannan sugar-positive ratio; however, the *Dectin-1* mRNA expression level in rs7309123G/G genotype healthy carriers was significantly reduced, thus affecting the susceptibility of the IPA. Plantinga et al. studied the relationship between *Dectin-1* Y238 X gene polymorphism and hematopoietic stem cell transplantation (HSCT) patients with *Candida albicans* colonization and found that patients who underwent HSCT because of malignant hematologic diseases often had invasive fungal infections with high morbidity and lethality. However, in sequential investigations of rheumatoid arthritis, they obtained different results [20,35]. These data confirm that Dectin-1 plays a crucial role in the antifungal immune response and that its gene polymorphisms can affect the susceptibility to fungal infection-related diseases.

rs735239, rs735240, rs4804803, and rs2287886 are located in the promoter region of *DC-SIGN*, and studies have found that *DC-SIGN* promoter polymorphisms are associated

Table 4. Comparison the corneal trauma history cases with normal controls.

SNP	Genotype/Allele	Cases (n=109) N (%)		Contorls (n=220) N (%)	χ^2	*P*
		Corneal Trauma History (n=68)	No Corneal Trauma History (n=41)			
rs17206002	AA	55(80.9)	32(78.0)	184 (83.6)	0.874	0.646
	TA	13(19.1)	9(22.0)	36 (16.4)		
rs11053613	AA	51(75.0)	31(75.6)	169 (76.8)	0.107	0.948
	GA	17(25.0)	10(24.4)	51 (23.2)		
rs3901532	AA	1(1.5)	1(2.4)	6(2.7)	0.429	0.980
	GA	25(36.8)	16(39.0)	81(36.8)		
	GG	42(61.8)	24(59.5)	133(60.5)		
rs3901533	GG	1(1.5)	1(2.4)	5(2.3)	0.235	0. 994
	GT	26(38.2)	16(39.0)	82(37.3)		
	TT	41(60.3)	24(58.5)	133(60.5)		
rs4804803	CC	0(0.0)	1(2.4)	5(2.3)	3.074	0. 546
	CT	12(17.6)	5(12.2)	44(20.0)		
	TT	56(82.4)	35(85.4)	171(77.7)		
rs2287886	CC	7(10.3)	7(17.1)	23 (10.5)	4.137	0.388
	TC	32(47.1)	14(34.1)	81(36.8)		
	TT	29(42.6)	20(48.8)	116(52.7)		
rs735239	AA	48(70.6)	25(61.0)	176(80.0)	9.446	0.051
	GA	17(25.0)	13(31.7)	40(18.2)		
	GG	3(4.4)	3(7.3)	4(1.8)		
rs735240	AA	7(10.3)	5(12.2)	9(4.1)	7.346	0.119
	AG	24(35.3)	12(29.3)	66(30.0)		
	GG	37(54.4)	24(58.5)	145(65.9)		

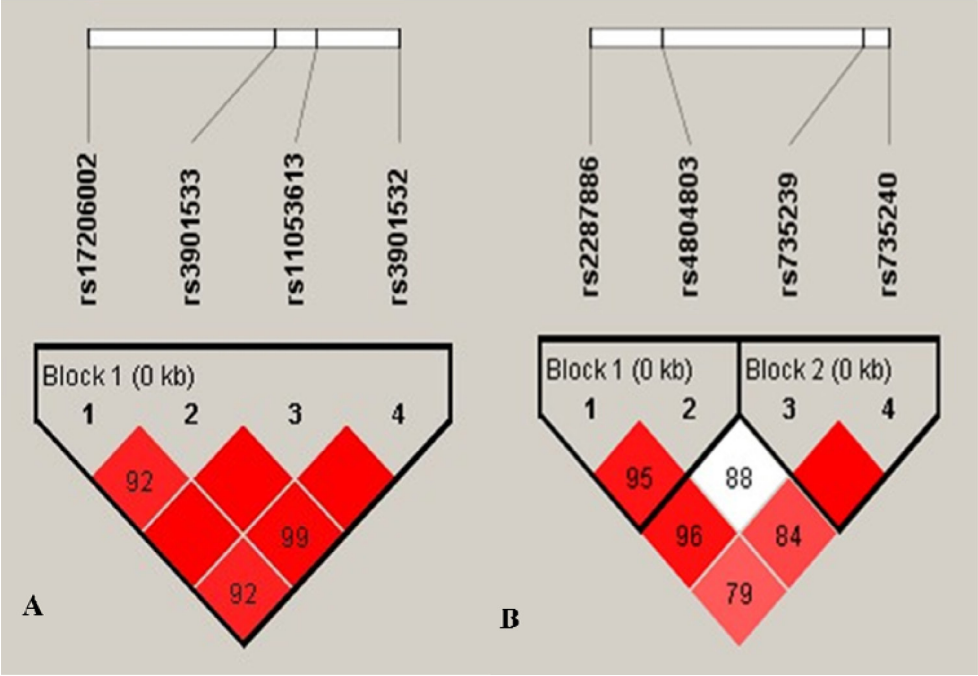

Figure 1. Linkage disequilibrium structure of the SNPs and haplotype blocks analyzed by Haploview 4.2 software. The D' value of each SNP pair is expressed as a percentage and shown within the respective square (D'= 1 not shown). Higher D' values are indicated in brighter red. **A**: LD plot of four SNPs of the *DC-SIGN* gene in 109 study subjects. The four SNPs constitute two haplotype blocks, with substantial LD among the SNPs of both blocks (block 1: D' =0.95; block 2: D'=1.00). **B**: LD plot of four SNPs of the *Dectin-1* gene in 109 study subjects. The four SNPs constitute a haplotype block spanning 0.2 kb of the *Dectin-1* gene, and the magnitude of LD between each SNP was extremely high, with pair-wise D' ≥0.92.

Table 5. Haplotype analysis for *DC-SIGN* gene polymorphisms.

No	Haplotype		Frequency			OR (95% CI)	*P*
			Total	Cases	Controls		
	rs4804803	rs2287886					
H1	T	T	0.691	0.656	0.709	0.728 (0.553–1.107)	0.165
H2	T	C	0.198	0.257	0.168	1.710 (1.154–2.534)	0.007
H3	C	C	0.107	0.083	0.120	0.657 (0.375–1.152)	0.140
	rs735239	rs735240					
H1	A	G	0.781	0. 725	0. 809	0.621 (0.425–0.909)	0.014
H2	G	A	0.137	0.193	0.109	1.949 (1.242–3.059)	0.003
H3	A	A	0.082	0.083	0.082	1.010 (0.560–1.823)	0.974

Table 6. Haplotype analysis for *Dectin-1* gene polymorphisms.

No	Haplotype				Frequency			OR (95% CI)	*P*
	rs17206002	rs3901533	rs11053613	rs3901532	Total	Cases	Controls		
H1	A	T	A	G	0.670	0.665	0.673	0.966(0.685–1.364)	0.845
H2	A	G	A	A	0.123	0.110	0.130	0.831(0.501–1.380)	0. 475
H3	A	T	G	G	0.112	0.115	0.111	1.034(0.620–1.724)	0.899
H4	T	G	A	A	0.081	0.096	0.075	1.1315(0.741–2.332)	0.348

with certain infectious diseases [30,36]. We speculated that although these SNPs in the promoter region of *DC-SIGN* could not change the encoded amino acid sequence, they might affect promoter activity and change the expression efficiency of *DC-SIGN*, thereby affecting the host antifungal immune reactions and prognosis process. Our results show that *DC-SIGN* SNPs are related to FK as a risk factor. But the alleles and genotypes frequencies of rs4804803 and rs2287886 and TT/CC haplotypes have no significant correlation with this disease. We suggest, therefore, that Allele C is a mutated loci and a different LD in the promoter region may interfere with transcription and affect the DC-SIGN-mediated recognition of fungal pathogens. This is potentially important as it suggests that a therapy designed to counteract the functional susceptibility of *DC-SIGN* may be beneficial even when administered to mature individuals. The promoter region can protect the RNA from RNAase, improve the efficiency of transcription to mRNA, and affect the accuracy of translation. After ruling out factors such as age, gender, and disease severity, we found there was no association between SNPs and FK. Typical FK patients usually have an agricultural corneal trauma before the occurrence of fungal infection [4,5,37,38]; however, in our study, some patients who were without history of corneal trauma were diagnosed with FK. Our results demonstrate that there is no genetic difference between patients with and without a corneal trauma history and suggest that other risk factors for FK exist. This is an interesting puzzle to be explored in future studies. In addition, further experimental research is necessary to define the direct functional association between *DC-SIGN* polymorphism and the occurrence of FK.

Researchers have found that some *DC-SIGN* SNPs have correlations with *Aspergillus*, *Mycobacterium tuberculosis*, dengue virus, and hepatitis B virus infections. Juan Sainz et al. found that *DC-SIGN* rs2287886 A allele carriers showed a depressed trend of invasive pulmonary *Aspergillus* infection [19]. A study on tick-borne encephalitis patients in Russia reported that among patients who had central nervous system diseases, the frequencies of the rs2287886 AA homozygote and the A allele were increased compared with other groups [39]. In several studies on dengue virus infection in Asia, -336A /G (rs4804803) polymorphism in *DC-SIGN* (CD209) promoter has association with dengue disease and dengue hemorrhagic fever. The rs4804803 A/G genotype can affect the expression of *DC-SIGN* on the cell surface to enhance the immune response and reduce viral replication [40,41]. *DC-SIGN* SNPs are reported to be associated with a risk of Kawasaki disease in Taiwanese children, celiac disease in in the Spanish population, human immunodeficiency virus infection, tuberculosis, severe acute respiratory ayndromes, human cytomegalovirus infection and hepatitis C virus infection [42-48]. Among these findings, some results seem to differ as a result of race differences.

In summary, our data revealed that the SNPs of *DC-SIGN* influence the susceptibility of FK in the northern Han Chinese population. In interpreting our findings, the present study has certain methodological limitations, such as the small sample size and adoption of multiple test corrections. Since our subjects were all recruited from the northern Han Chinese population, the result might be affected by geographic, ethnic, and genetic background factors. However, the lack of a relationship between *Dectin-1* and the pathogenesis of FK is not definitive from this study. Because there are several mutations in these genes, further replication studies on the genetic pathogenesis of FK in diverse ethnic groups are needed to confirm the importance of *DC-SIGN* and *Dectin-1* polymorphisms in affecting the risk of FK. In conclusion, more replication studies with a larger sample size in different populations and further functional investigations are necessary to elucidate the mechanisms of *DC-SIGN* and *Dectin-1* polymorphisms on the susceptibility of FK and to lead to novel pharmacological treatments for FK.

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