Molecular Vision 2015; 21:948-954 <http://www.molvis.org/molvis/v21/948>
Received 31 May 2015 | Accepted 22 August 2015 | Published 25 August 2015

© 2015 Molecular Vision

Possible association of killer cell immunoglobulin-like receptor genotypes and haplotypes with dry eye disease in a Han Chinese population

Guifang Ren,[1] Huiyu Li,[2] Wenben Qiao,[3] Hua Shen,[3] Yunlong Zhuang,[3] Tiejun Shao,[1] Haipeng Hu,[1] Xiuping Zhang[1]

[1]Department of Ophthalmology, The 4th People's Hospital of Jinan, Shandong Province, P. R. China; [2]University of Minnesota Twin Cities, Minneapolis MN; [3]Blood Center of Shandong Province, Shandong Province, P. R. China

Purpose: The objective of this study was to explore whether killer immunoglobulin-like receptor (*KIR*) genotypes and haplotypes are associated with dry eye disease (DED) in a Han Chinese population.
Methods: Polymerase chain reaction with sequence-specific primers (PCR-SSP) method was used to genotype *KIR* genes in 106 patients with DED and 220 healthy controls.
Results: Twenty-three *KIR* genotypes were observed in the DED patient and healthy control groups, ten of which had not been described previously. The genotype G and haplotype 4 were associated with increased risk of DED, and the odds ratio (OR) and 95% confidence interval (95% CI) were 2.58, 1.10–6.02 and 2.48, 1.31–4.69, respectively; while haplotype 2 appeared to have an inverse association with the disease (OR, 0.64; 95% CI, 0.44–0.92). Genotype B/B was also associated with increased risk of DED, and the OR and 95% CI were 2.35 and 1.09–5.10, respectively. *KIR* haplotypes A and B have distinctive centromeric (*Cen*) and telomeric (*Tel*) gene-content motifs, and *Cen*-B/B was associated with increased risk of DED (OR, 2.38; 95% CI, 1.03–5.49). However, all frequencies of these *KIR* genotypes and haplotypes were no longer statistically significant between the two groups after the Bonferroni correction was applied for multiple testing.
Conclusions: There was a possible association between certain *KIR* genotypes and haplotypes with DED in a Han Chinese population. However, additional confirmation is required.

Dry eye disease (DED) is one of the most common ocular disorders, affecting 10–30% of the population [1]. Patients with DED experience visual disturbance, eye dryness, irritation, foreign body sensation, light sensitivity, itching, and even blindness [2,3].

Recent studies have shown that DED is an inflammatory disease that shares similar characteristics with autoimmune diseases [4-6], although the exact pathogenesis of DED remains unclear. The immunological responses of the host have been supposed to play a critical role in the clearance of inflammation, including cytokine production, antigen participation, and receptor recognition, which depends on the host's genetic background.

Killer immunoglobulin-like receptor (KIR) molecules are encoded by the *KIR* gene family, which exhibit genetic variation in the number and type of the genes presented on a haplotype [7]. The *KIR* haplotypes can be resolved into two broad haplotypes termed A and B, which consist of four conserved framework genes, *KIR2DL4* (ID 3805; OMIM 604945), *3DL2* (ID 3812; OMIM 604947), *3DL3* (ID 115653; OMIM 610095), and *3DP1* (ID 548594; OMIM 610604). Haplotype B contains a variety of genes, including *KIR2DL2* (ID 3803; OMIM 604937), *2DL5* (ID 100125575; OMIM 605305), *2DS1* (ID 3806; OMIM 604952), *2DS2* (ID 100132285; OMIM 604953), *2DS3* (ID 3808; OMIM 604954), *2DS5* (ID 3810; OMIM 604956), or *3DS1* (ID 3813; OMIM 604946), while haplotype A is characterized by the absence of all these genes. *KIR* genes are found in two adjacent clusters where the framework genes flank each cluster: *KIR3DL3* and *3DP1* flank the centromeric (*Cen*) cluster while *KIR2DL4* and *3DL2* flank the telomeric (*Tel*) cluster [7]. *KIR* gene motifs are defined based on the combination of *KIR* genes present in the *Cen* or *Tel* segment on the haplotype, and *KIR* haplotypes A and B, with different distributions among ethnic groups, have distinctive *Cen* and *Tel* gene-content motifs [8-10].

Natural killer (NK) cells are a vital component of the innate and adaptive immune systems, expressing several cell surface receptors (KIRs, C-type lectin superfamily receptors, and natural cytotoxicity receptors) [11]. NK cell activity is regulated by an extensive repertoire of regulatory receptors including the most polymorphic KIR family [12]. Several studies in *KIR* gene diversity were associated with infectious and noninfectious diseases [12,13]. KIR molecules

Correspondence to: Guifang Ren, Department of Ophthalmology, The 4th People's Hospital of Jinan, 50 Shifan Road, Jinan city 250031, Shandong province, P.R. China; Phone: 86-531-81313304; FAX: 86-531-89269968; email: guifangren@gmail.com

play an important role in anti-inflammation immunity by providing activating or inhibitory signals to regulate the activation of NK cells and T cell subsets [13,14]. The *KIR* genotype with distinctive gene content from maternal and paternal haplotypes produces diversity among individuals, which may influence the individuals' immunity to different diseases. Interestingly, the associations between diseases and *KIR* genotypes have been observed in several clinical studies [14-16]. For example, individuals with *KIR* genotype A/A were reported to be relatively protected against psoriatic vulgaris and idiopathic bronchiectasis [14,15], and individuals with genotype A/B were significantly more likely to remain seronegative among long-term HIV-exposed subjects than those with genotype A/A [16]. Previously, we showed that *KIR2DS2* was associated with the susceptibility to severe DED [17]. However, until now, the role of the overall *KIR* genotype in patients with DED has remained unclear. The aim of this study was to investigate the distribution of *KIR* genotypes and haplotypes in a Han Chinese population of 106 patients with DED and 220 healthy controls with polymerase chain reaction with sequence-specific primers (PCR-SSP).

METHODS

Subjects: A total of 106 patients with DED, consisting of 47 men and 59 women, were recruited from the Ophthalmology Department and 220 unrelated healthy subjects from the health examination center of the 4th People's Hospital of Jinan as a control group described by Ren et al. [17]. Of the 106 patients, 27 were classified with moderate DED and 79 (31 male, 48 female) with severe DED. These DED patients had no other diseases. Briefly, the patients with DED were diagnosed by Schirmer's I test, which was advised by the National Eye Institute workshop. A 35.5-mm filter paper strip was used to measure the amount of tears that were produced over a period of 5 min. More than 10-mm wetting of the filter paper was normal. Patients with moderate dry eyes had wetting values between 10 and 5 mm. Patients with severe dry eyes had wetting values of less than 5 mm. Meanwhile, healthy subjects had wetting values of more than 10 mm in Schirmer's I test. Valid informed consent was obtained from each participant, and this study was approved by the human ethics committee of the 4th People's Hospital of Jinan. And the study adhered to the tenets of the Declaration of Helsinki and the ARVO statement on human subjects.

KIR genotyping: KIR genotyping was performed using PCR-SSP in all samples collected from the recruited subjects for the following 16 *KIR* genes: *2DL1–5*, *3DL1–3*, *2DS1–5*, *3DS1*, *2DP1*, and *3DP1*. The PCR sequence-specific polymorphism primers used for the detection of *KIR* genes, and the PCR amplification methods were previously described [17]. Briefly, 20–50 ng DNA was amplified in 10 ml volume containing 0.2 mM dNTP, 0.5U Taq DNA polymerase (Promega Corporation), 0.4 mM primers (except for *KIR2DS1*, 0.8 mM), and 1× PCR buffer. PCR amplificationwas carried out in a 9700 thermal cycler (PerkinElmer) under the following conditions: initial denaturing at 94 °C for 4min, followed by 30 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 90 s, plus a final extension at 72 °C for 10 min. Partial annealing temperatures were changed as follows: *KIR2DS2* (63 °C), *KIR2DS3* (63 °C), *KIR2DS4* (61 °C), and *KIR2DS5* (63 °C).

Genotype and haplotype analysis: Each genotype was given the putative haplotype combination according to the model described by Hsu et al. [18]. In assigning genes to a specific haplotype, the following assumptions were made: 1) All haplotypes contained *KIR3DL3*, *2DL4*, and *3DL2*; 2) the haplotypes contained either *2DL2* or *2DL3*, but not both; and 3) the haplotypes contained either *3DP1* or *3DP1* variant (*3DP1v*), but not both [18]. In the assessment of the *KIR* haplotypes, haplotype B was defined by the presence of one or more of the following genes: *KIR2DL5*, *2DS1*, *2DS2*, *2DS3*, *2DS5*, and *3DS1*. Conversely, haplotype A was defined by the absence of all these genes [19]. Four conserved framework genes divide the *KIR* locus into similarly sized *Cen* and *Tel* segments that differ in gene content [8]. Genotypes for the *Cen* and *Tel* parts of the *KIR* locus were defined according to Cooley et al.'s description [8]. Briefly, *Cen*-A/A contains *2DL3* only, *Cen*-A/B contains *2DL3* with *2DS2* and/or *2DL2*, and *Cen*-B/B contains *2DS2* and/or *2DL2* but no *2DL3*, whereas *Tel*-A/A has *3DL1* and *2DS4* only, *Tel*-A/B has *3DL1* and *2DS4* with *3DS1* and/or *2DS1* and *Tel*-B/B lacks *3DL1* and/or *2DS4*.

Statistical analysis: Genotype frequencies were determined by directly counting the number of individuals who were positive in some particular *KIR* phenotype specificity. Each genotype was given the putative haplotype combination according to the model described by Hsu et al. [18]. A chi-square was used to test for the statistical significance of the genotypes or haplotypes between the DED patient group and the healthy control group. P values less than 0.05 were regarded as statistically significant. The strength of the association was estimated by calculating the odds ratio (OR) and the 95% confidence interval (95% CI). Statistical analysis was performed using the SPSS 15.0 (SPSS Inc., Chicago, IL) software package. Bonferroni correction for multiple testing was applied in the comparison of the genotype and haplotype frequencies between the two groups. The corrected p values (*P*c) are shown in the tables.

RESULTS

KIR genotype frequencies in control subjects and patients: In our study, all tested *KIR* genes were detected in the healthy control and DED patient groups. Framework genes (*KIR2DL4*, *3DL2*, *3DL3*, and *3DP1*) were observed in all individuals. All *KIR* genotypes and putative haplotypes were determined according to Hsu et al.'s description [18].

In this study, no significant differences in the frequencies of the *KIR* genotypes and haplotypes were found between the patients with moderate DED and the healthy controls with the chi-square analysis (data not shown). However, certain *KIR* genotypes and haplotypes were associated with increased or reduced risk of severe DED (Table 1 and Table 2). Twenty-three *KIR* genotypes were observed in both groups, including ten new genotypes of NF1-NF10, which has not been observed in Caucasians thus far [18]. Among these genotypes, 20 were determined in healthy controls and 21 in patients with DED (Table 1). In the healthy controls, the three *KIR* genotypes with higher frequencies in rank order were AJ (37.73%), AH (11.36%), and AF (10.45%). In the patients with DED, genotypes AJ (27.85%), G (13.92%), and P (10.12%) were the three higher genotypes. Genotype G was associated with increased risk of DED, and the OR and 95% CI were 2.58 and 1.10–6.02, respectively. However, the frequency of genotype G was not statistically significant between the two groups after the Bonferroni correction was applied for multiple testing. The other *KIR* genotypes did not show any associations between the two groups.

KIR haplotype frequencies in control subjects and patients: In this study, all 23 *KIR* genotypes were resolved into corresponding pairs of haplotypes (Table 2). There were 16 different haplotypes of which haplotype 2 was the most common (56.14%), followed by haplotype 1 (15.91%) and 5

Table 1. The frequencies of *KIR* genotype in healthy controls and DED patients.

Genotype	Haplotype	Healthy controls		DED patients		OR(95%CI)	p value	*Pc* (n=23)
		+	gf(%)	+	gf(%)			
E	1,3	2	0.91	1	1.27	1.40(0.13–15.63)	0.785	
F	1,4	3	1.36	2	2.53	1.88(0.31–11.46)	0.487	
G	4,5	13	5.91	11	13.92	2.58(1.10–6.02)	0.025*	>0.05
H	2,4	5	2.27	3	3.8	1.70(0.40–7.27)	0.471	
I	5,8	1	0.45	1	1.27	2.81(0.17–45.43)	0.448	
M	2,8	2	1.04	2	2.53	2.83(0.39–20.45)	0.282	
P	2,17	22	10	8	10.12	1.01(0.43–2.38)	0.974	
AE	1,6	8	3.64	2	2.53	0.69(0.14–3-31)	0.64	
AF	1,2	23	10.45	6	7.59	0.70(0.28–1.80)	0.461	
AG	1,1	9	4.09	3	3.8	0.93(0.24–3.51)	0.909	
AH	2,5	25	11.36	6	7.59	0.64(0.25–1.63)	0.346	
AI	1,5	16	7.27	4	5.06	0.68(0.22–2.10)	0.5	
AJ	2,2	83	37.73	22	27.85	0.64(0.36–1.12)	0.115	
NF1	2,9	1	0.45	1	1.27	2.81(0.17–45.43)	0.448	
NF2	4,13	1	0.45	1	1.27	2.81(0.17–45.43)	0.448	
NF3	2,6	1	0.45	0	0	-	0.548	
NF4	4,8	1	0.45	1	1.27	2.81(0.17–45.43)	0.448	
NF5	2,11	2	0.91	0	0	-	0.395	
NF6	2,23	0	0	1	1.27	-	0.095	
NF7	6,6	0	0	1	1.27	-	0.095	
NF8	1,4	0	0	1	1.27	-	0.095	
NF9	?,?	1	0.45	1	1.27	2.81(0.17–45.43)	0.448	
NF10	?,?	1	0.45	1	1.27	2.81(0.17–45.43)	0.448	

+, positive numbers; gf, genotype frequency; OR, odds ratio; 95% CI, 95% confidence interval; *, indicates statistical significance (p<0.05) according Chi Square analysis; *Pc*, corrected p value after Bonferroni correction; ?, unclassed haplotype.

Table 2. The frequencies of *KIR* haplotypes in healthy controls and DED patients.

Haplotype	Healthy controls		DED patients		OR(95%CI)	p value	P_C (n=13)
	+	hf(%)	+	hf(%)			
1	70	15.91	22	13.92	0.86 (0.51–1.44)	0.553	
2	247	56.14	71	44.94	0.64(0.44–0.92)	0.016*	>0.05
3	2	0.45	1	0.63	1.40(0.13–15.49)	0.786	
4	23	5.23	19	12.03	2.48(1.31–4.69)	0.004*	>0.05
5	55	12.5	22	13.92	1.13(0.67–1.93)	0.647	
6	9	2.05	4	2.53	1.24(0.38–4.10)	0.719	
8	4	0.91	4	2.53	2.83(0.70–11.46)	0.128	
9	1	0.23	1	0.63	2.80(0.17–44.97)	0.449	
11	2	0.45	0	0	-	0.396	
13	1	0.23	1	0.63	2.80(0.17–44.97)	0.449	
17	22	5	8	5.06	1.01(0.44–2.32)	0.975	
23	0	0	1	0.63	-	0.095	
?	4	0.91	4	2.53	2.83(0.70–11.46)	0.128	

+, positive numbers; hf, haplotye frequency; OR, odds ratio; 95% CI, 95% confidence interval; *, indicates statistical significance (p<0.05) according Chi Square analysis; *Pc*, corrected p value after Bonferroni correction; ?, unclassed haplotype.

(13.92%) in the healthy controls and the patients with DED. Haplotype 4 was associated with increased risk of DED, and the OR and 95% CI were 2.48 and 1.31–4.69, respectively; while haplotype 2 appeared to have an inverse association with the disease (OR, 0.64; 95% CI, 0.44–0.92). However, the frequencies of haplotypes 4 and 2 were not statistically significant between the two groups after the Bonferroni correction was applied for multiple testing. The other *KIR* haplotypes did not show any associations between the two groups.

The distributions of *KIR* haplotype A and B are shown in Table 3. The frequency of haplotype A was higher than haplotype B in the healthy controls and the patients with DED. Genotype B/B was associated with increased risk of DED, and the OR and 95% CI were 2.352 and 1.09–5.10, respectively. Interestingly, when compared the distributions of the *Cen* and *Tel* segments of the *KIR* genotype in the two groups (Table 4), *Cen*-B/B was also associated with increased risk of DED (OR, 2.380; 95% CI, 1.03–5.49). However, the frequencies of genotype B/B and *Cen*-B/B were not statistically significant between the two groups after the Bonferroni correction was applied for multiple testing. The other *Cen* and *Tel* motifs of the *KIR* genotypes did not show any associations between the two groups.

DISCUSSION

The *KIR* gene system spans a region of about 150 kb on chromosome 19 [11]. The number and type of *KIR* genes arranged on the haplotypes vary greatly, which produces substantial differences in *KIR* gene content among individuals [7]. Genetic diversity within the *KIR* locus can moderate the NK cells and T cell subset responses to a diverse array of diseases and thus suggests that *KIR* diversity may influence susceptibility or resistance to different diseases [20]. Recent studies showed that *KIR* genotypes are associated with autoimmune diseases such as idiopathic bronchiectasis [15] and psoriasis vulgaris [14] and microbial pathogen diseases caused by hepatitis B virus [21], Ebola virus [22], and HIV [16]. However, little is known about the association of *KIR* genotypes with DED, which is characterized by sustained inflammation on the ocular surface. Interestingly, this is the first study to analyze *KIR* genotypes associated with DED.

In our study, patients with DED and healthy controls were identified as having *KIR* genotype A/A, A/B, or B/B based on the multiple *KIR* genes the individuals possessed. Similar distributions of *KIR* genotypes were observed between the two groups (Table 3). Interestingly, individuals with genotype B/B had an increased risk of severe DED, which was consistent with, to some extent, our previous report that *KIR2DS2* was associated with susceptibility to DED [17] since genotype B/B contains *KIR2DS2*. It was similar to the results that genotypes with haplotype B were found to

Table 3. The frequencies of *KIR* genotypes A/A, A/B and B/B and haplotypes A and B, observed in healthy controls and DED patients.

Genotyping	Healthy controls		DED patients		OR(95%CI)	p value	P_C
	+	%	+	%			
KIR genotypes		gf		gf			(n=3)
A/A	116	52.73	35	44.3	0.71(0.43–1.20)	0.199	
A/B	87	39.55	31	39.24	0.99(0.58–1.67)	0.962	
B/B	17	7.73	13	16.46	2.35(1.09–5.10)	0.027*	>0.05
KIR haplotypes		hf		hf			(n=2)
A	319	72.5	101	63.92	0.67(0.46–0.98)	0.043*	>0.05
B	121	27.5	57	36.08	1.49(1.01–2.19)	0.043*	>0.05

+, positive numbers; gf, genotype frequency; hf, haplotye frequency; OR, odds ratio; 95% CI, 95% confidence interval; *, indicates statistical significance (p<0.05) according Chi Square analysis; *Pc*, corrected p value after Bonferroni correction; ?, unclassed haplotype.

be increased in Japanese psoriasis vulgaris cases [14]. The structurally distinctive characteristic of haplotype B is the multiplicity of activating *KIRs* that might increase chances of accidental activation of immunocytes over the threshold. Moreover, this inaccurate activation would occur especially in patients who do not possess the proper combinations of inhibitory *KIR*s [14]. Recent data supported the hypothesis that DED is a localized autoimmune disease resulting from the imbalance between protective immunoregulatory and proinflammatory mechanisms [6]. Combined with our data, these findings supported previously proposed models of *KIR*-mediated autoimmunity [23].

Dividing these *KIR* haplotypes into the *Cen* and *Tel* motifs showed that homozygosity for *Cen*-B/B was also associated with increased risk of DED (Table 4). Interestingly, it was recently shown that *Cen*-B/B was associated with a lower risk of relapse after unrelated transplantation for acute myelogenous leukemia [8]. These data suggested that individuals with the *Cen*-B/B motif might generate different immune responses to different diseases.

We further refined *KIR* genotypes [18] to analyze the association between patients with DED and the healthy controls. In our study, ten new genotypes NF1–10 that have not been observed in Caucasians thus far were identified [18]. The distinctive distributions of the *KIR* genotypes between

Table 4. The frequencies of *KIR* centromeric (*Cen*) and telomeric (*Tel*) gene content motifs in healthy controls and EDE patients.

Motifs[#]	Healthy controls		DED patients		OR (95% CI)	p value	P_C (n=6)
	+	gf(%)	+	gf (%)			
Centromeric (*2DS2*, *2DL2*, *2DL3*)							
Cen-A/A	163	74.09	56	70.89	0.85(0.48–1.51)	0.581	
Cen-A/B	43	19.55	12	15.19	0.74(0.37–1.48)	0.391	
Cen-B/B	14	6.36	11	13.92	2.38(1.03–5.49)	0.037*	>0.05
Telomeric (*3DL1*, *3DS1*, *2DS1*, *2DS4*)							
Tel-A/A	149	67.73	51	64.56	0.87(0.51–1.49)	0.608	
Tel-A/B	38	17.27	17	21.52	1.31(0.69–2.49)	0.403	
Tel-B/B	33	15	11	13.92	0.92(0.44–1.91)	0.817	

#: KIR centromeric (Cen) and telomeric (Tel) gene content motifs were defined according to the description of Cooley et al. [8]. Briefly, Cen-A/A contains *2DL3* with *2DS2* and/or *2DL2*, and Cen-B/B contains *2DS2* and/or *2DL2* but no *2DL3*, whereas Tel-A/A has *3DL1* and *2DS4* only, Tel-A/B has *3DL1* and *2DS4* with *3DS1* and/or *2DS1* and Tel-B/B lacks *3DL1* and/or *2DS4*. +, positive numbers; gf, genotype frequency; OR, odds ratio; 95% CI, 95% confidence interval; *, indicates statistical significance (p<0.05) according Chi Square analysis; *Pc*, corrected p value after Bonferroni correction; ?, unclassed haplotype.

Han Chinese and Caucasian populations suggested the role of the natural selection of certain genes over the others. Interestingly, our data in Table 1 and Table 2 show that genotype G and haplotype 4 were associated with increased risk of DED, whereas haplotype 2 appeared to have an inverse association with the disease. These data suggested that different *KIR* genes within a genotype/haplotype might use the combinations of synergistic receptors to mediate different natural cytotoxicity here. Lu et al. [24] reported that individuals with genotype M or FZ1 or haplotype 4 had an increased risk of hepatitis B virus infection, whereas individuals with genotype AH or haplotype 5 facilitated the clearance of the hepatitis B virus. These data suggested that different *KIR* genotypes/haplotypes might contribute to the different immune responses to autoimmune and virus infectious diseases and that certain *KIR* gene combinations could provide an immune microenvironment to get rid of or be susceptible to different diseases.

All frequencies of these *KIR* genotypes and haplotypes were no longer significant between the healthy controls and the patients with DED after the Bonferroni correction analysis. The Bonferroni correction is a critical statistical method for mitigating the risk of making false-positive conclusions, and appropriate use can ensure the integrity of studies in which a large number of significance tests are used, although it tends to be a bit too conservative and increases the risk of generating false negatives [25].

Our findings might help develop biomarkers to monitor the ocular surface inflammatory status and provide a new interesting therapeutic strategy for considering the molecular basis of DED, which will not only improve our knowledge to fully understand the mechanisms that lead to DED but also provide directions for developing effective and safe anti-inflammatory treatments that will be beneficial for patients with DED.

In conclusion, our study showed that *KIR* genotype G, haplotype 4, and *Cen*-B/B were possibly associated with increased risk of DED, whereas haplotype 2 was possibly associated with a reduced risk of DED in a Han Chinese population. These studies should be subjected to multivariate analysis and repeated in patients of other ethnic origins from the same and different regions.

ACKNOWLEDGMENTS

The authors thank Dr. Roberta Greenwood for critically reading and improving the English manuscript. This work was supported by Shandong Province Young and Middle-Aged Scientists Research Awards Fund (BS2011SW044), Medicine and Health Science and Technology Development Planning Projects of Shandong Province (2013WS0170).

REFERENCES

1. Gayton JL. Etiology, prevalence, and treatment of dry eye disease. Clin Ophthalmol 2009; 3:405-12. [PMID: 19688028].
2. Miljanović B, Dana R, Sullivan DA, Schaumberg DA. Impact of dry eye syndrome on vision-related quality of life. Am J Ophthalmol 2007; 143:409-15. [PMID: 17317388].
3. Chen Y, Chauhan SK, Saban DR, Sadrai Z, Okanobo A, Dana R. Interferon-γ-secreting NK cells promote induction of dry eye disease. J Leukoc Biol 2011; 89:965-72. [PMID: 21402771].
4. Luo L, Li DQ, Doshi A, Farley W, Corrales RM, Pflugfelder SC. Experimental dry eye stimulates production of inflammatory cytokines and MMP-9 and activates MAPK signaling pathways on the ocular surface. Invest Ophthalmol Vis Sci 2004; 45:4293-301. [PMID: 15557435].
5. Solomon A, Dursun D, Liu Z, Xie Y, Macri A, Pflugfelder SC. Pro-and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. Invest Ophthalmol Vis Sci 2001; 42:2283-92. [PMID: 11527941].
6. Yagci A, Gurdal C. The role and treatment of inflammation in dry eye disease. Int Ophthalmol 2014; 34:1291-301. [PMID: 25416345].
7. Zhuang YL, Song Y, Zhu C, Zhang Y, Wang D, Nie X, Liu Y, Ren GJ. Association of *KIR* genotypes and haplotypes with syphilis in a Chinese Han population. Scand J Immunol 2012; 75:361-7. [PMID: 22126195].
8. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, Marsh SG, Geraghty D, Spellman S, Haagenson MD, Ladner M, Trachtenberg E, Parham P, Miller JS. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood 2010; 116:2411-9. [PMID: 20581313].
9. Uhrberg M, Parham P, Wernet P. Definition of gene content for nine common group B haplotypes of the Caucasoid population: *KIR* haplotypes contain between seven and eleven *KIR* genes. Immunogenetics 2002; 54:221-9. [PMID: 12136333].
10. Shilling HG, Young N, Guethlein LA, Cheng NW, Gardiner CM, Tyan D, Parham P. Genetic control of human NK cell repertoire. J Immunol 2002; 169:239-47. [PMID: 12077250].
11. Shastry A, Sedimbi SK, Rajalingam R, Rumba I, Kanungo A, Sanjeevi CB. Different KIRs confer susceptibility and protection to adults with latent autoimmune diabetes in Latvianand Asian Indian populations. Ann N Y Acad Sci 2008; 1150:133-8. [PMID: 19120281].
12. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol 2005; 5:201-14. [PMID: 15719024].

13. Carrington M, Martin MP. The impact of variation at the *KIR* gene cluster on human disease. Curr Top Microbiol Immunol 2006; 298:225-57. [PMID: 16329188].

14. Suzuki Y, Hamamoto Y, Ogasawara Y, Ishikawa K, Yoshikawa Y, Sasazuki T, Muto M. Genetic polymorphisms of killer cell immunoglobulin-like receptors are associated with susceptibility to psoriasis vulgaris. J Invest Dermatol 2004; 122:1133-6. [PMID: 15140215].

15. Boyton RJ, Smith J, Ward R, Jones M, Ozerovitch L, Wilson R, Rose M, Trowsdale J, Altmann DM. HLA-C and killer cell immunoglobulin-like receptor genes in idiopathic bronchiectasis. Am J Respir Crit Care Med 2006; 173:327-33. [PMID: 16254274].

16. Jennes W, Verheyden S, Demanet C, Adjé-Touré CA, Vuylsteke B, Nkengasong JN, Kestens L. Cutting edge: resistance to HIV-1 infection among African female sex workers is associated with inhibitory KIR in the absence of their HLA ligands. J Immunol 2006; 177:6588-92. [PMID: 17082569].

17. Ren G, Shao T, Zhuang Y, Hu H, Zhang X, Huang J, Liu Y, Liu D. Association of killer cell immunoglobulin-like receptor and human leukocyte antigen-C genotype with dry eye disease in a Chinese Han population. Genet Test Mol Biomarkers 2012; 16:910-4. [PMID: 22509813].

18. Hsu KC, Liu XR, Selvakumar A, O'Reilly RJ, Dupont B. Killer Ig-like receptor haplotype analysis by gene content: evidence for genomic diversity with a minimum of six basic framework haplotypes, each with multiple subsets. J Immunol 2002; 169:5118-29. [PMID: 12391228].

19. Marsh SG, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D, Vilches C, Carrington M, Witt C, Guethlein LA, Shilling H, Garcia CA, Hsu KC, Wain H. Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. Hum Immunol 2003; 64:648-54. [PMID: 12770798].

20. Kulkarni S, Martin MP, Carrington M. The Yin and Yang of HLA and KIR in human disease. Semin Immunol 2008; 20:343-52. [PMID: 18635379].

21. Gao X, Jiao Y, Wang L, Liu X, Sun W, Cui B, Chen Z, Zhao Y. Inhibitory KIR and specific HLA-C gene combinations confer susceptibility to or protection against chronic hepatitis B. Clin Immunol 2010; 137:139-46. [PMID: 20643584].

22. Wauquier N, Padilla C, Becquart P, Leroy E, Vieillard V. Association of KIR2DS1 and KIR2DS3 with fatal outcome in Ebola virus infection. Immunogenetics 2010; 62:767-71. [PMID: 20878400].

23. Nelson GW, Martin MP, Gladman D, Wade J, Trowsdale J, Carrington M. Cutting edge: heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis. J Immunol 2004; 173:4273-6. [PMID: 15383555].

24. Lu Z, Zhang B, Chen S, Gai Z, Feng Z, Liu X, Liu Y, Wen X, Li L, Jiao Y, Ma C, Shao S, Cui X, Chen G, Li J, Zhao Y. Association of *KIR* genotypes and haplotypes with susceptibility to chronic hepatitis B virus infection in Chinese Han population. Cell Mol Immunol 2008; 5:457-63. [PMID: 19118512].

25. Napierala MA. What Is the Bonferroni Correction? AAOS Now 2012; 6:40-5. .

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 25 August 2015. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.