Genetic profiling of ocular inflammation: further evidence for a gender-specific association of *C5* with uveitis

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ABSTRACT

Objective: Uveitis is a major cause for visual impairment, our previous studies have made significant advancements in depicting the genetic profile of complement genes in uveitis. This study aimed to further investigate whether the terminal pathway gene, *Complement component 5* (*C5*), confers susceptibility to uveitis.

Methods: Six tagging SNPs in *C5* were genotyped in 592 unrelated study subjects: 141 anterior uveitis patients, 158 patients with non-infectious intermediate and posterior uveitis, and 293 controls. Multiple in-depth analyses have been conducted.

Results: Among the six *C5* SNPs, rs17611 was significantly associated with uveitis after adjusted for gender and SNP-gender interaction ($P = 0.017$). After stratification by gender, rs17611 G allele and GG homozygosity confers an increased risk for uveitis in males ($P = 0.004$, OR = 1.67 and $P = 0.009$, OR = 2.69, respectively) but not in females. Moreover, genotype-phenotype correlation analysis revealed an association between rs17611 and disease recurrence ($P = 0.045$). The haplotype GC, defined by rs17611 and rs2269066, was also found to be associated with total uveitis and specific intermediate and posterior uveitis subtype ($P = 0.0055$, OR = 1.51 and $P = 0.0084$, OR = 1.58, respectively). Other polymorphisms were not significantly associated with either investigated uveitis entities.

Conclusions: This study shows a gender-specific association of *C5*-rs17611 with uveitis, indicating that *C5* may have an epistatic effect with gender in the pathogenesis of uveitis. This study help us depict the disease profile and estimate the contribution of each complement activation pathway in ocular immunologic process.

INTRODUCTION

Uveitis is a major cause for visual impairment, which is a group of heterogeneous ocular inflammatory diseases with complex phenotypes [1]. Uveitis is classified by the anatomical location of the inflammation and also whether it is caused by an infectious agent [2, 3]. Anterior uveitis (AU), which refers to inflammation of the iris and ciliary body, is the most common form in clinics. The intermediate and posterior uveitides comprise a group of ocular disorders that may occur in isolation, as part of a panuveitis, or in conjunction with systemic disease, including Vogt–Koyanagi–Harada disease (VKH), Behçet's disease (BD), and sarcoidosis [4]. Uveitis predominantly affects people of working age between 20–50 years old and can be a devastating sight-threatening condition resulting from several complications, including cystoid macular edema (CME), secondary glaucoma, secondary cataract, vitreous opacities and optic neuropathy. The current treatment of uveitis contains

immunomodulatory agents aimed at first controlling acute inflammation and subsequent keeping long-term remission. Despite considerable progress in treatment, safe and effective management is still a clinical challenge. The exact pathogenesis is still unclear in many forms of uveitis, but accumulating evidence has demonstrated that uveitis is an inflammatory condition and mainly mediated by various endogenous immunological mechanisms [5]. Moreover, genetic factors as well as immune imbalance are potential cofactors implicated in the initiation and development of the disease [6–9].

The complement system is a key component of innate immunity and is involved in several inflammatory conditions including uveitis. Complement activation occurs via three distinct pathways, referred to as the classical, alternative, and lectin pathways [10, 11]. An imbalance in complement, either by insufficient or excessive complement activation, can have important pathological consequences [12]. Recent *in vivo* study revealed that activation of complement system is critical for the development of experimental autoimmune anterior uveitis (EAAU), conversely, depletion of the host's complement system could result in complete inhibition of EAAU [13, 14].

The Figure 1 shows activation of the complement system and some key regulators/genes implicated in uveitis. Our research team have made great efforts in depicting the genetic profile of uveitis and intraocular inflammation regarding complement pathway genes, several genes have been extensively investigated and identified to be associated with many forms of uveitis, these genes included complement factor H (*CFH*), complement factor B (*CFB*), complement factor I (*CFI*), and CD59 [15–20]. Meanwhile, we have largely ruled out the involvement of some complement factors in the disease genetics, such as component 1 inhibitor (*C1INH*), and complement component 3 (*C3*) [21, 22]. Notably, some genetic associations between AU and complement genes were also found in NIPU patients in our study cohort, suggesting that these two uveitis entities shared general genetic background although presenting different clinical phenotypes. Meanwhile, the genetic variants in *CFH* and *CFB*, involved in the alternative pathway, were also found to be associated with type 2 diabetic retinopathy (T2DM) in our previous study, viewing from the perspective of inflammation [23].

These results together suggested that complement activation, especially the alternative pathway and "upstream" factors, play a predominate role in the pathogenesis of uveitis, as well as in other intraocular inflammations.

The complement component 5 (C5), being the first of many components of the terminal pathway, mediates many potent inflammatory and cytolytic events and plays a major role in the complement system [24]. Recent studies have demonstrated that C5 cleavage contributed to the full expression of experimental autoimmune uveoretinitis (EAU), and selective C5 blockade via systemic and local routes of administration could suppress uveitis [25]. Furthermore, *C5* gene has been linked to affect susceptibility to several immunologic diseases, including AMD, rheumatoid arthritis, and bronchial asthma [26–28]. So far however, little is known about the genetic profile of *C5* in uveitis. Taking above together, we herein aimed to investigate whether *C5* variants, involved in the final pathway of the complement cascade, are associated with uveitis, with a view to constructing an overall genetic profile of complement activation in uveitis.

METHODS

Study participants

A total of 299 uveitis patients, including 141 patients with AU and 158 patients with intermediate and posterior uveitis were enrolled in the present case-control study. The following data were obtained from all patients: gender, age at presentation, age onset, number of flares, recurrent frequency, and prevalence of severe ocular complications. All patients of uveitis were given detailed clinical and ophthalmic assessments. The diagnosis of AU was based on the SUN criteria, All AU were recruited during the active phase of uveitis and followed for at least two years after recruitment [3]. Acute AU was defined as AU resolving completely within 3 months, chronic AU as AU not fully resolved within 3 months, and recurrent AU as the development of AU more than once. Considering intermediate and posterior uveitis may share an underlying immune etiology, we combined them to investigate the genetic impact as a whole, named NIPU. These patients were categorized into three specific subtypes: IU, VKH and Behçet's disease. Patients with any of the following situations were excluded (1) Patients with uveitis secondary to ocular or systemic infections; (2) patients who were unable to cooperate during ocular examination and with chronic uveitis at the onset of the study. The mean age of the global uveitis patients is 48.6 ± 14.5 years. The control group consisted of 293 unrelated, healthy individuals without major eye diseases or any systemic immune-related disorders. The mean age of the control individuals was significantly greater than that of uveitis patients as expected, this is because we purposely recruited subjects older than 55 years as controls to largely reduce the confounding effects from younger subjects.

Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study. All the procedures were conducted according to the tenets of the Declaration of Helsinki. The study

protocol was approved by Ethnic Committee on Human Research, Harbin Medical University.

Single nucleotide polymorphism selection and genotyping

Tag SNPs across *C5* region were obtained from HapMap Project database for the Han Chinese population (http://hapmap.ncbi.nlm.nih.gov/). Six SNPs (rs1017119, rs10985126, rs1548782, rs17611, rs2269066 and rs12237774) were selected by the tagger-pairwise method. The 6 tagging SNPs evaluated in this study captured 100% alleles in the *C5* locus with MAF larger than 0.1 and a mean r^2 of 0.95. Venous blood was obtained from each study subject and genomic DNA was extracted by using a DNA blood kit (QIAamp; Qiagen, Hilden, Germany). These 6 SNPs were genotyped by *Taq*Man genotyping assays on an ABI Prism 7000 Sequence Detection System according to the manufacturer's protocol (Applied Biosystems [ABI], Foster City, CA). The genotypes were read by the system software (Prism 7000 SDS software version 1.1; ABI). More details were described in our previous studies [20, 22].

Statistical analysis

Hardy-Weinberg Equilibrium (HWE) of individual SNP in the control group was tested using by χ^2 test. Allelic or genotype association of each SNP was evaluated using the chi-square test or Fisher's exact test (SPSS, version 20.0; SPSS Inc., Chicago, IL). The odds ratio (OR) and corresponding 95% confidence interval (CI) were estimated with the minor allele as reference. Pairwise linkage disequilibrium (LD, *D'*) and EM-based haplotype association analysis were assessed using the Haploview software. Logistic regression analysis was performed to adjust the effect of SNPs with gender, referring to previously reported. We stratified the study subjects according to subtype, gender and performed association analysis of the SNP in each gender stratum. $P < 0.05$ was considered statistically significant. *P* values were corrected by Bonferroni test for multiple comparisons (n = total number of SNPs), or permutation test in Haploview software. In particular, since the SNP rs17611 was analyzed separately in males and females, a *P* value of less than 0.025 (= 0.5/2) was considered statistically significant.

RESULTS

The clinical characteristics of the study subjects are shown in Table 1. This study involved 592 unrelated Han Chinese participants: 141 with AU (47.2%), 158 with NIPU (52.8%), and 293 healthy controls. NIPU, as a mixed disease entity, is comprised of 51 (32.3%) VKH, 45 (28.5%) IU and 62 (39.2%) BD.

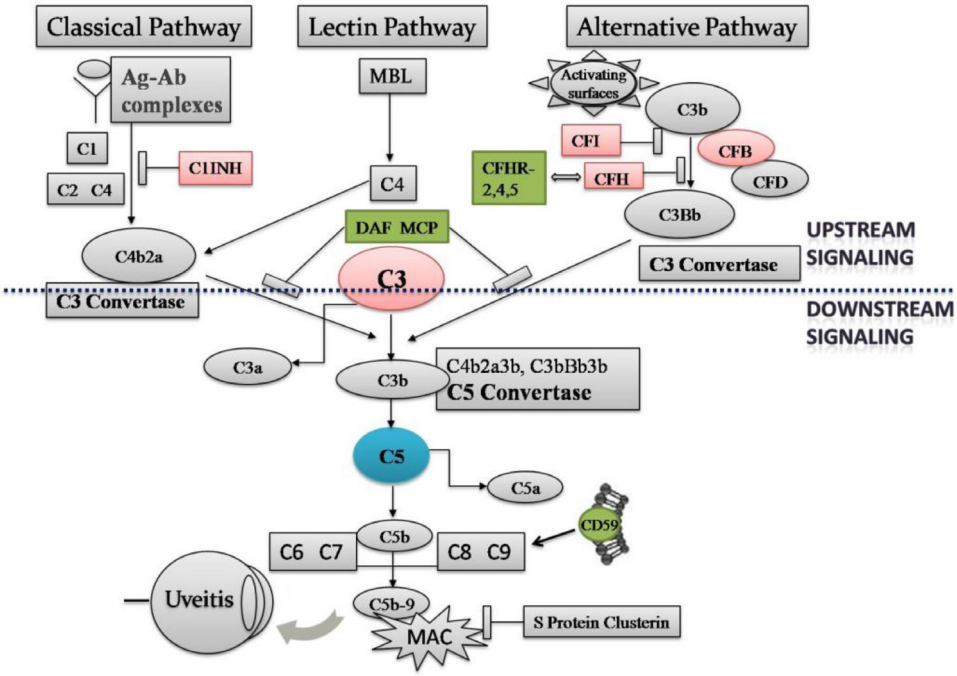

Figure 1: Simplified diagram of complement system and some key regulators implicated in uveitis. Previously investigated genes (highlighted in red background); C5 gene investigated in the present study (highlighted in blue background); Other complement factors/genes implicated in uveitis needs further investigation (highlighted in green background). Abbreviations: C, complement component; C1INH, C1 inhibitor; DAF, decay accelerating factor; F, factor (eg, factor B, factor H); CFHR, the factor H related family; MBL, mannan-binding lectin; MAC, membrane attack complex; CD59, MAC-inhibitory protein.

Table 1: The clinical characteristics of the study subjects

	AU (n = 141)	NIPU (n = 158)			Total Uveitis (n = 299)	Control (n = 293)	Comparison	
		VKH (n = 51)	IU (n = 45)	BD (n = 62)			AU vs. NIPU	Total vs. Control
Gender (Male/Female)	72/69	22/29	16/29	42/18	152/147	129/164	0.94	0.097
Mean age ± SD(years)								
general	50.4 ± 14.6	49.0 ± 16.3	41.3 ± 14.9	49.5 ± 11.2	48.6 ± 14.5	74.3 ± 7.5	0.042	$P < 0.001$
male	50.2 ± 14.4	55.5 ± 16.3	42.3 ± 15.4	50.2 ± 11.6	50.1 ± 14.3	73.7 ± 7.0	0.095	$P < 0.001$
female	50.6 ± 14.9	43.9 ± 14.6	40.8 ± 14.8	47.8 ± 10.5	47.0 ± 14.7	74.8 ± 7.8	0.005	$P < 0.001$
Age range(years)								
general	11–87	20–81	18–73	25–69	11–87	55–94	NA	NA
male	18–87	20–81	18–72	25–69	18–87	55–89	NA	NA
female	11–87	23–73	18–73	26–60	11–87	55–94	NA	NA

AU: anterior uveitis; NIPU: non-infectious intermediate and posterior uveitis; VKH: Vogt–Koyanagi–Harada disease.
IU: intermediate uveitis; BD: Behçet's disease; NA: not applicable; SD: standard deviation.

As a slight tendency toward a higher proportion of males was observed in the global uveitis group (P = 0.097) compared with controls, gender was adjusted in the following association analysis by using logistic regression. Among AU patients, 55 (39.0%) were HLA-B27 positive and 86 (61.0%) were HLA-B27 negative. Because we purposely recruited subjects older than 55 years as controls to avoid including patients with early-onset disease, the mean age of the control individuals was significantly greater than that of all uveitis patients as expected (all $P <$ 0.001). The mean age of the AU was significantly higher than that in NIPU patients (P = 0.042), the difference was obvious in the female subgroup (P = 0.005). Among NIPU patients, the mean age of VKH was significantly higher than that of IU in general and male subgroups in particular (one-way ANOVA Fisher LSD, P = 0.024 and 0.01, respectively).

All of the tested SNPs followed HWE in the control group ($P > 0.05$). In single marker analysis, none of the 6 SNPs showed a significant allelic association with uveitis, although there was a trend towards higher proportion of rs17611/G allele in global uveitis and NIPU subtype compared with controls (P = 0.035 and 0.047, respectively), the P value could not withstand the Bonferroni correction ($P_{corr} > 0.05$, Table 2). Likewise, no SNP showed a significant association with the disease or its subtypes in any genetic models if a conservative Bonferroni correction applied . (Table 3).

In the epistatic analysis, no SNP*SNP interaction was detected between each *C5* SNP and *CFH* rs800292, *CFH* rs1065489, or *CFB* rs1048709 (data not shown). However, logistic regression analysis revealed that *C5* rs17611 was significantly associated with the global uveitis patients after adjusted for gender and SNP-gender interaction (P = 0.017, Table 4). Stratification by gender revealed that rs17611 was significantly associated with uveitis in males but not in females (Table 5). The rs17611/G allele showed a risk effect toward uveitis in males (P = 0.004, OR = 1.67, 95% CI: 1.18–2.38). In the genotypic analysis, rs17611 showed a significant association with uveitis in male patients under recessive model (P = 0.009, OR = 2.69, 95% CI: 1.25–5.77). On the contrast, this SNP was not associated with female uveitis patients in any genetic models ($P > 0.025$). Such gender different susceptibility was not observed in uveitis subtypes (data not shown).

Regarding AU, since the interaction between HLA-B27 and complement genes in uveitis has been found in our previous studies, we thus performed stratification analysis according to HLA-B27 status. The results showed no interaction of *C5* variants with HLA-B27 (Table 6). Since NIPU comprises a group of ocular disorders, stratification analysis was performed according to its subtypes, with a view to indentifying specific disease-association. The results showed no significant associations between *C5* variants and NIPU subtypes (data not shown).

Genotype-phenotype correlation analysis in terms of multiple clinical features were evaluated, such as age at presentation, age onset, number of flares, recurrent frequency, as well as the presence of posterior synechiae (PS) and keratic precipitates (KP). We discovered a relationship between *C5*-rs17611 and recurrence. The risk GG genotype is correlated with a higher recurrence frequency (P = 0.045, Figure 2). Associations with other clinical features were not observed.

Pairwise LD analysis was performed across the *C5* locus by using these 6 SNPs, One haplotype block was detected in total uveitis entity and its two subtypes (rs17611/G and rs2269066/C, Figure 3). The haplotype GC, defined by the two SNPs, showed a significant risk for total uveitis patients and NIPU subtype (P = 0.0055, permutation P = 0.033, OR = 1.51, 95% CI: 1.12–2.04; P = 0.0084, permutation P = 0.044, OR = 1.58, 95% CI: 1.11–2.24, respectively). A borderline association was also detected in AU patients, but the difference could not

Table 2: Allelic association of SNPs in C5 genes with AU, NIPU and total uveitis

Variation	Location	Minor allele	Allele Distribution (%)				Allelic Association					
			AU (n = 141)	NIPU (n = 158)	Total Uveitis (n = 299)	Control (n = 293)	AU vs. Control		NIPU vs. Control		Total Uveitis vs. Control	
							p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
rs1017119	122765792	C	0.15	0.09	0.12	0.14	0.77	1.06 (0.71–1.59)	0.014	0.57 (0.36–0.90)	0.18	0.79 (0.56–1.11)
rs10985126	122776839	C	0.23	0.22	0.23	0.24	0.70	0.94 (0.67–1.31)	0.48	0.89 (0.64–1.23)	0.50	0.91 (0.70–1.19)
rs1548782	122809021	T	0.19	0.22	0.20	0.20	0.60	1.10 (0.77–1.58)	0.59	0.91 (0.65–1.27)	0.97	0.99 (0.75–1.32)
rs17611	122809663	G	0.41	0.42	0.42	0.36	0.145	0.81 (0.60–1.08)	0.047	0.75 (0.57–0.99)	0.035	0.78 (0.62–0.98)
rs2269066	122823755	T	0.21	0.21	0.21	0.22	0.75	1.06 (0.75–1.50)	0.74	1.06 (0.76–1.48)	0.69	1.06 (0.80–1.40)
rs12237774	122847359	T	0.16	0.20	0.18	0.19	0.17	1.31 (0.89–1.91)	0.86	0.97 (0.69–1.37)	0.49	1.11 (0.83–1.49)

AU: anterior uveitis; NIPU: non-infectious intermediate and posterior uveitis; C5: complement component 5; CI: confidence interval.
OR: odds ratio.

Table 3: Genotypic association of SNPs in C5 genes with AU, NIPU and Total Uveitis

Variation	Genotype	Genotype Distribution (%)				Genetic model	Genotype Association		
		AU (n = 141)	NIPU (n = 158)	Total Uveitis (n = 299)	Control (n = 293)		AU vs. Control	NIPU vs. Control	Total Uveitis vs. Control
							P-value	P-value	P-value
rs1017119	TT/CT/CC	2/38/101	1/25/132	3/63/233	5/73/215	Dominant	0.70	0.014 0.54(0.33–0.89)	0.20
						Recessive	1.0*	0.67*	0.50*
rs10985126	TT/CT/CC	6/53/82	4/62/92	10/115/174	19/104/170	Dominant	0.98	0.97	0.97
						Recessive	0.35	0.07	0.077
rs1548782	AA/AT/TT	6/41/94	12/45/101	18/86/195	9/101/183	Dominant	0.39	0.76	0.49
						Recessive	0.58*	0.036* 2.59 (1.07–6.30)	0.086
rs17611	AA/AG/GG	26/63/52	32/70/56	58/133/108	37/135/121	Dominant	0.38	0.23	0.20
						Recessive	0.11	0.032 1.76 (1.05–2.95)	0.025 1.67 (1.06–2.61)
rs2269066	CC/CT/TT	7/44/90	6/53/99	13/97/189	10/106/177	Dominant	0.49	0.64	0.48
						Recessive	0.44	0.83	0.56
rs12237774	CC/CT/TT	3/38/100	7/49/102	10/87/202	9/96/188	Dominant	0.16	0.93	0.38
						Recessive	0.76*	0.46	0.85

AU: anterior uveitis; NIPU: non-infectious intermediate and posterior uveitis; C5: complement component 5; *Fisher exact test.

Table 4: Logistic regression analysis of C5 SNPs, gender and SNP*gender interaction

SNPs	P value for SNP effect	P value for gender effect	P value for SNP*gender effect
rs1017119	0.20	0.21	0.32
rs10985126	0.19	0.36	0.47
rs1548782	0.49	0.55	0.70
rs17611	0.017	0.42	0.15
rs2269066	0.61	0.19	0.24
rs12237774	0.56	0.27	0.36

Table 5: Association of C5-rs17611 with Uveitis stratified by gender

SNP ID	Gendern	Allele Distribution (%)			*P*-value	Odds Ratio (95% CI)	Genotype Distribution (%)			*P*-value	Odds Ratio (95% CI)
			Global Uveitis	Control			Global Uveitis		Control		
rs17611	Male	G	125 (41.1)	76 (29.5)	0.004	1.67 (1.18–2.38)	GG	28 (18.4)	10 (7.8)	0.032†	1.68 (1.04–2.72)
		A	179 (58.9)	182 (70.5)			AG	69 (45.4)	56 (43.4)	0.009‡	2.69 (1.25–5.77)
							AA	55 (36.2)	63 (48.8)		
	Female	G	124 (42.2)	133 (40.5)	0.68	1.07 (0.78–1.47)	GG	30 (20.4)	27 (16.5)	0.90†	0.97 (0.61–1.54)
		A	170 (57.8)	195 (59.5)			AG	64 (43.5)	79 (48.2)	0.37‡	1.30 (0.73–2.31)
							AA	53 (36.1)	58 (35.4)		

C5: complement component 5; CI: confidence interval.
†*P* values in dominant genetic models.
‡*P* values in recessive genetic models.

Table 6: Comparison of allele frequencies of C5 polymorphisms in patients with AU versus control subjects stratified by HLA-B27 status

Variation	Minor Allele	HLA-B27 Positive AU	HLA-B27 Negative AU	Controls	*P*-value§	*P*-value£
		(*n* = 55)	(*n* = 86)	(*n* = 293)		
rs1017119	C	0.16	0.14	0.14	NS	NS
rs10985126	C	0.21	0.24	0.24	NS	NS
rs1548782	T	0.22	0.17	0.20	NS	NS
rs17611	G	0.40	0.41	0.36	0.39	0.10
rs2269066	T	0.20	0.21	0.22	NS	NS
rs12237774	T	0.14	0.17	0.19	0.15	NS

Data are the number of subjects (% of the total group); §*P*-value for HLA-B27-Positive Patients versus Controls; £*P*-value for HLA-B27-Negative Patients versus Controls; NS Not significant

remain after permutation test ($P = 0.048$ permutation $P > 0.05$, Table 7).

DISCUSSION

In the present study, we have, for the first time, performed a haplotype tagging SNP analysis of the *C5* gene concurrently in two different clinical uveitis entities, AU and NIPU. The six tagging SNPs capture 100% of common genetic variations in the *C5* locus. Our results demonstrated a strongly gender-specific association of rs17611 with susceptibility to uveitis. Meanwhile, we also identified a novel risk haplotype block across the *C5* gene. In addition, genotype–phenotype correlation

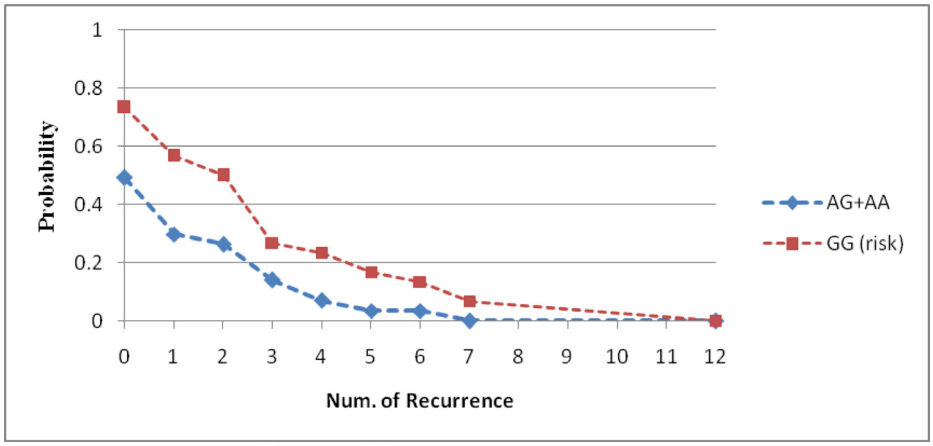

Figure 2: The correlation between C5-rs17611 genotypes and uveitis recurrence.

analysis revealed that rs17611 might be a clinical maker related to recurrence, its GG genotype predisposed to a higher recurrent frequency. These findings suggest that *C5* may play a crucial role in uveitis genetics. Different gender susceptibility and SNP-gender interaction suggest additional risk factors should be required for *C5* to exert its effect in the pathogenesis of ocular inflammation, likely through an epistatic function.

The complement system is a part of the immune system that is involved in uveitis and other immune mediated diseases. As described above, previous reports from our laboratory have established a critical role of complement activation in uveitis. Several complement genes were extensively investigated, particular attention was paid on the "upstream" functional pathways. Of them, variants in the *CFH*, *CFB* and *CFI* involved in the alternative pathway, were identified as genetic risk markers for various uveitis subtypes [16–20]. A recent report from our laboratory also demonstrated that the classical pathway gene, *C1INH*, as well as the central component of the cascade, *C3* gene, may confer either no or limited risk for uveitis susceptibility [21, 22]. Overall, our findings together suggest that the alternative pathway as well as the "upstream" cascade of complement system confers major genetic impact on uveitis susceptibility. However, the genetic studies on the role of complement "downstream" pathway in uveitis have not yet been reported. The object of the current study was therefore to examine the genetic predisposition of the terminal pathway gene *C5* in uveitis. The terminal complement pathway comprises the final five components of the complement cascade, C5 through to C9. A critical event in the cascade is the cleavage of C5 into fragments C5a and C5b, and the subsequent formation of the membrane attack complex (C5b-9) which is involved with cell migration, cytokine release, and production of potent inflammatory mediators [29]. There is experimental evidence that C5 cleavage contributes to the full expression of EAU, and that selective C5 blockade via systemic and local routes of administration can suppress the disease [25]. There is also molecular evidence that polymorphisms of *C5* are associated with several autoimmune or inflammatory disorders, such as AMD, rheumatoid arthritis and bronchial asthma [26–28]. These studies suggested that C5 plays an important role in immune-related disorders via enhanced production of specific cytokines. Results of this study indicated that *C5* rs17611 is a risk factor for male uveitis, which enrich our knowledge of the genetic architecture of complement genes in uveitis up to now. Meanwhile, study by Chai L *et al*, also reported that rs17611 was associated with periodontitis [30]. Furthermore, functional analysis on rs17611 showed that individuals homozygously expressing the risk allele G exhibit increased C5a and decreased C5 in plasma, which potentially explain the genetic association of *C5* rs17611 with uveitis [31].

Some interesting findings of our study are the gender-dependent effect of *C5* gene in uveitis. Actually, such gender differences in the genetic profiles have also been described in our previous studies and others, where the *CFH* rs800292 was found as a genetic risk marker for AU in Chinese females [18]. Similarly, in the study of Liu *et al*, a male-specific association of *C3* gene with polypoidal choroidal vasculopathy (PCV) was identified [32]. Our finding that *C5* rs17611 was associated with uveitis only in males suggests that the *C5* gene is likely to be a risk factor for the male predominance of uveitis, although the exact mechanism and how complement genes interact with gender in these disease pathogenesis remain unclear. The gender specificity might account for different pathway of complement activation in inflammation [33]. It might also be related to the small number of subjects, the wide variety of uveitis syndromes, or the distinct complex regulatory mechanism for immune-related diseases [34].

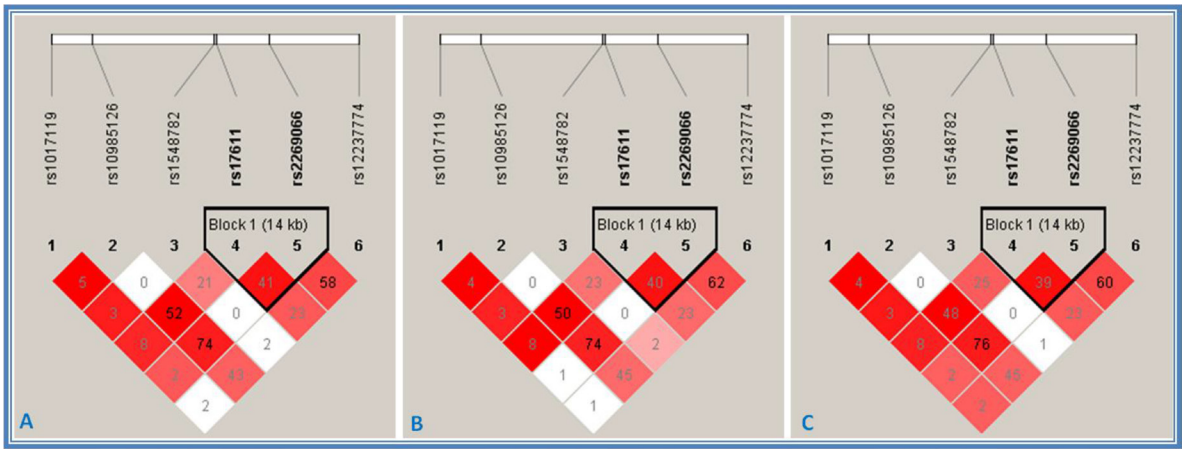

Figure 3: Linkage disequilibrium (LD) structure of the C5 locus for AU (**A**), NIPU (**B**) and Total Uveitis (**C**) LD was measured using data from all controls, total uveitis and its subtypes. The haplotype block was defined by the confidence interval method implemented in the Haploview software. The LD (r^2) between any two SNPs is listed in the cross cells. AU: anterior uveitis; NIPU: non-infectious intermediate and posterior uveitis

Table 7: Haplotype association of C5 gene with uveitis and its subtypes

Block rs17611-rs2269066	Frequency				Association (*P*-value) (Permutation test)		
	AU	NIPU	Total uveitis	Control	AU vs. Control	NIPU vs. Control	Total uveitis vs. Control
A-C	0.591	0.575	0.583	0.636	0.21	0.075	0.063
G-T	0.205	0.205	0.205	0.207	0.94	0.94	0.92
G-C	0.203	0.219	0.211	0.149	0.048 (0.23)	0.0084 (0.044)	0.0055 (0.033)

AU: anterior uveitis; NIPU: non-infectious intermediate and posterior uveitis; C5: complement component 5; Pcorr association analysis results from permutation test (iterations 10,000)

Given the significance of rs17611, correlations between this SNP and clinical course of inflammation were evaluated. The data showed a relationship between rs117611 and recurrence, its GG genotype predisposed a higher recurrent frequency. The small sample size, the diversified phenotypes of uveitis and the limited follow-up periods might affect the genotype-phenotype correlations. Nevertheless, our results provided novel understanding for the genetic impact on clinical severity.

Apart from that, several implementation-specific analyses have been performed. The interaction of HLA-B27 with *C5* in AU was not observed like previous studies [20]. Considering that NIPU present different clinical phenotypes and may have a different etiology, stratification analysis in NIPU subtypes (IU vs. Control and PU vs. Control) was also performed. We failed to find any genetic associations with specific subtypes, although the sample size within subsets was relatively small. A linkage established among complement genes will give more power to the results and provide deeper insight into the whole genetic profiles of uveitis, in regard to this, the epistatic analysis was performed and no SNP*SNP interaction was detected. The results suggest that C5 might be an independent susceptibility factor for uveitis.

Several limitations existed in this study. Firstly, the relatively small sample size, particularly in the subgroups, will reduce the statistical power and therefore some modest associations could not be detected. Secondly, we have only evaluated six *C5* tag-SNPs in this study and thus our findings will not reflect the disease risk of unexamined variants in this region. Moreover, the majority of uveitis is complex, polygenic diseases, and susceptible genes might likely have small independent effects. Last but not least, although significant advancements have been made in depicting the genetic profiles of uveitis from our laboratory, the function and molecular mechanism of these complement pathway genes in uveitis are largely unknown. Further in-depth studies involving large number of patients and multi cohorts to explore the genetic influences, as well as functional studies that focus on these complement genes in uveitis are warranted. Thus, these results should be interpreted cautiously.

In summary, this study shows a strongly gender-specific association of *C5* variant with uveitis. Together with our previous data, the overall genetic profile of uveitis regarding complement pathway genes suggest that the alternative pathway, as well as the terminal component of complement cascade contribute most to uveitis susceptibility. Our study provides new insights into the underlying pathogenic mechanisms of ocular inflammation, and may lead to novel potential therapeutic targets.

Author contributions

M.M.Y., and J.W. designed the experiments. X.Y.W., D.J.K. and Y.W.L performed the experiments. M.M.Y., L.D. and H.Y.S. performed the analysis and wrote the paper. J.J.F and X.H.Y. revised the paper. All authors contributed to the editing of the paper and to scientific discussions.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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