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LABORATORY STUDY

FPR1 interacts with *CFH*, *HTRA1* and smoking in exudative age-related macular degeneration and polypoidal choroidal vasculopathy

XY Liang[1], LJ Chen[1], TK Ng[1], J Tuo[2], J-L Gao[3], PO S Tam[1], TY Y Lai[1], C-C Chan[2] and CP Pang[1]

Abstract

Purpose **To determine the genetic association of an inflammation-related gene, formyl peptide receptor 1 (*FPR1*), in exudative age-related macular degeneration (AMD) and polypoidal choroidal vasculopathy (PCV).**

Methods **The coding region of *FPR1* gene was sequenced in 554 unrelated Chinese individuals: 155 exudative AMD patients, 179 PCV patients, and 220 controls. Interactions and combined effects of *FPR1* with complement factor H (*CFH*), high temperature requirement factor A1 (*HTRA1*), and smoking were also investigated.**

Results **A total of 28 polymorphisms in *FPR1* were identified. Single nucleotide polymorphisms (SNP) rs78488639 increased the risk to exudative AMD ($P = 0.043$) and PCV ($P = 0.016$), whereas SNP rs867229 decreased the risk to exudative AMD ($P = 0.0026$), but not PCV. Homozygous G allele of rs1042229 was associated with exudative AMD ($P = 0.0394$, odds ratio (OR) = 2.27, 95% confident interval: 1.08–4.74), but not with PCV. Exudative AMD, but not PCV, was associated with the heterozygous genotypes of rs2070746 ($P = 0.019$, OR = 0.57) and rs867229 ($P = 0.0082$, OR = 0.54). Significantly, interactions were identified among *FPR1* rs78488639, *CFH* rs800292, and *HTRA1* rs11200638 in both exudative AMD and PCV. Combined heterozygous risk alleles of *CFH* rs800292 GA and *FPR1* rs78488639 CA were posed to PCV ($P = 2.22 \times 10^{-4}$, OR = 10.47), but not exudative AMD. Furthermore, *FPR1* rs78488639 CA combining with *HTRA1* rs11200638 and smoking was also predisposed risks to exudative AMD and PCV.**

Conclusion ***FPR1* is associated with exudative AMD and PCV in a Hong Kong Chinese cohort. *FPR1* rs78488639 interacted with *CFH* rs800292, *HTRA1* rs11200638, and smoking, enhancing risk to exudative AMD and PCV.**

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[1]Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, Hong Kong Eye Hospital, Kowloon, Hong Kong

[2]Laboratory of Immunology, National Eye Institute, National Institutes of Health, Bethesda, MD, USA

[3]Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, USA

Correspondence:
CP Pang, Department of Ophthalmology and Visual Sciences, The Chinese University of Hong Kong, 4/F, Hong Kong Eye Hospital, 147K Argyle Street, Kowloon, Hong Kong
Tel: +85 2 3943 5801;
Fax: +85 2 2715 9490.
E-mail: cppang@cuhk.edu.hk

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Introduction

Age-related macular degeneration (AMD) is an irreversible, progressive, and sight-threatening macular disease in the elderly population, affecting over 50 million individuals worldwide.[1] Clinically, the late stage of AMD can be divided into two subgroups: geographic atrophy and exudative/neovascular forms.[2] Exudative AMD involves choroidal neovascularization (CNV), which is an ingrowth of new vascular vessels from choriocapillaries beneath retinal pigment epithelium (RPE) arising toward subretinal space and the neuroretina. These new vessels have greater tendency of leakage and bleeding.[3] CNV invades into the retina via the Bruch's membrane and RPE, leading to serous or hemorrhagic detachments of either the RPE or neuroretina. Consequently, this leads to subretinal fibrosis[4] and ultimately irreversible

blindness. The exudative form accounts for >80% of significant rapid visual loss from AMD.[5,6]

Inflammation has an influential role in exudative AMD.[7] Drusen, the hallmark of AMD, contain inflammatory components, such as complement activators and inhibitors, membrane attack complex as well as inflammasome.[8] Local RPE cell damage by aging activates and recruits choroidal dendritic cells, contributing to the formation of drusen and CNV.[9] The choroidal infiltrate is also composed of macrophages and lymphocytes.[10,11] The activated inflammatory cells secrete collagenase and elastase, which erode the thinned Bruch's membrane and facilitate migration of choroidal capillaries.[12] Moreover, interaction between inflammatory cells and vascular endothelial growth factor (VEGF) also indicates the role of inflammation in CNV development.[13] In addition, significant association of inflammatory markers with AMD suggests that inflammation could participate in AMD pathogenesis.[14,15]

As inflammation is involved in AMD pathogenesis, inflammation-related genes should be studied in AMD patients. The formyl peptide receptor 1 (*FPR1*) gene, located on chromosome 19q13.4,[16] encodes a seven transmembrane domain G protein-coupled receptor. It is expressed mainly by mammalian phagocytic leukocytes and involved in inflammatory responses by activation of chemotatic peptides.[17] This receptor allows phagocytic cells to recognize the presence of exogenous organisms, such as bacteria, and mediates the traffic of phagocytes to the sites of tissue damage.[18] Therefore, *FPR1* is a crucial molecule in innate immunity. Moreover, its ligand, the N-formyl peptides, is produced only by bacteria and mitochondrial proteins in nature.[19] Mitochondrial dysfunction is also associated with AMD pathogenesis.[20] Hence, we hypothesized that *FPR1* could have a positive role in AMD pathogenesis.

Polypoidal choroidal vasculopathy (PCV) shares many similarities in clinical features with exudative AMD. But they differ in clinical course, imaging feature, response to treatment, and prognosis.[21] In order to identify the role of *FPR1* in exudative AMD, the whole-coding region of *FPR1* gene was sequenced in this study. In addition, the *FPR1* sequence was screened in PCV and compared with that of exudative AMD. Furthermore, the interactions and combined effects of *FPR1* with complement factor H (*CFH*) and high temperature requirement factor A1 (*HTRA1*) were also determined as *CFH* and *HTRA1* are associated with both exudative AMD and PCV.[22,23]

Materials and methods

Study subjects

A total of 554 participants were recruited at the Prince of Wales Hospital Eye Centre, including 155 exudative AMD patients, 179 PCV patients and 220 age-matched control subjects (Supplementary Table 1). All study subjects were given complete ophthalmic examinations. AMD was graded according to an international classification and grading system.[24] Patients with exudative AMD had non-drusenoid RPE detachment, choroidal neovascularization, serous or hemorrhagic retinal detachments, subretinal or sub-RPE hemorrhage, or fibrosis. The diagnosis of PCV was distinguished from exudative AMD by fluorescein angiography and indocyanine green angiography (ICGA).[25] PCV patients had subretinal red or orange nodules and hemorrhagic pigment epithelial detachment and characteristic sacculated vascular abnormalities in the inner choroid as visualized on ICGA. The control subjects were recruited from elderly people >60 years of age. According to the complete ophthalmic examinations, the control subjects did not have any identifiable signs of AMD, PCV, or other retinal or optic nerve diseases except for mild senile cataract and refractive errors. Smoking habits were also recorded. A smoker was defined as a person who had smoked at least five cigarettes daily for >1 year. The study subjects were divided into two groups: those who had never smoked, those who were ex-smokers, and current smokers. The study protocol, approved by the Ethics Committee for Human Research at the Chinese University of Hong Kong, is in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from each study subject.

Sequence analysis

Genomic DNA was extracted (Qiagen QIAamp DNA Blood Mini kit, Qiagen, Hiden, Germany) from peripheral blood samples following the supplier's instructions. All samples were screened for sequence alterations in the entire coding region (exon 2) and intron–exon junctions of *FPR1* (ENSG00000171051) by PCR with specific primer sets (Supplementary Table 2), followed by direct DNA sequencing (BigDye Terminator Cycle Sequencing Reaction Kit, v3.1, Applied Biosystems, Foster City, CA, USA) on a DNA sequencer (ABI 3130XL, Applied Biosystems). In addition, all samples were genotyped for *CFH* rs800292 and *HTRA1* rs11200639 using direct DNA sequencing with specific primers.[22,23]

Amino-acid substitution analysis

For the non-synonymous variants identified in *FPR1*, functional effects of the amino-acid substitutions were evaluated and predicted by two web-based analyzing programs: PolyPhen (polymorphism phenotyping, http://genetics.bwh.harvard.edu/pph/, provided by

the Bork Group and the Sunyaev Lab, Brighamand Women's Hospital, Harvard Medical School, Boston, MA, USA) and SIFT (Sorting Intolerant from Tolerant, http://sift.jcvi.org/, provided in the public domain by the J Craig Venter Institute, Rockville, MD, USA). The PolyPhen score > 1.0 would be considered as 'probably damaging' and otherwise would be considered as 'benign'. The SIFT score ≤ 0.05 is predicted to be damaging, and > 0.05 is predicted to be tolerated.

Statistical analysis

All the identified polymorphisms were assessed for Hardy–Weinberg equilibrium in controls using χ^2 analysis. Allelic and genotypic distributions were compared using the χ^2-test or Fisher's exact test (SPSS, version 16.0; SPSS Science, Chicago, IL, USA). Bonferroni's correction was used for multiple testing corrections. Linkage disequilibrium (LD) plots and haplotype-based association analyses were generated by Haploview (v4.2, http://www.broadinstitute.org/).[26] All associated polymorphisms in *FPR1* were assessed by logistic regression (SPSS). Logistic regression analysis included only gender as indicator because the recruited control subjects were older, excluding the potential patients with major late-onset ocular diseases. SNPs that remained significant after adjusting for other individual SNPs were selected for interaction and combined effect analyses. The pair-wise association of *FPR1* rs78488639 with *CFH* rs800292, *HTRA1* rs11200638 or smoking was evaluated using the χ^2-test or Fisher's exact test (SPSS).

Results

The gender distribution of PCV patients in our study was ~2 : 1 (male:female, 123 : 51), whereas that of exudative AMD patients (84 : 70) and control subjects (101 : 119) was ~1 : 1 (Supplementary Table 1). This was compatible to a previous study.[27]

A total of 28 single nucleotide polymorphisms (SNPs) were identified in *FPR1* (Table 1). Variants rs7253355 (c.1-342C > T) and c.513G > A (T171T) were excluded for further analysis as they violated Hardy–Weinberg equilibrium in the control subjects. Among the remaining 26 polymorphisms, 18 were rare SNPs (minor allele frequency <3%) and did not show significant association. They were also excluded for further analysis. The triallelic variant rs1042229 (c.576T > G/C, N192K/N) was analyzed independently. The homozygous of risk allele G was associated with exudative AMD ($P = 0.0394$, odds ratio (OR) = 2.27, 95% confident interval (CI): 1.08–4.74), but not with PCV ($P = 0.241$) or in comparison between exudative AMD and PCV ($P = 0.137$). Besides, the codon change caused by risk allele C was synonymous and did not show any association under either autosomal recessive or dominant model (data not shown). Thus, totally seven polymorphisms (c.117C > T, rs78488639, rs2070745, rs2070746, rs5030880, rs867228, and rs867229) were further analyzed (Table 1). SNP rs78488639 (c.289C > A, L97M) was associated with both exudative AMD ($P = 0.043$) and PCV ($P = 0.029$), whereas rs867229 (c.1053 + 196C > T) was associated with only exudative AMD ($P = 0.0026$). Genotypic difference between exudative AMD and PCV was detected in rs867229 ($P = 0.014$). Haplotype analysis revealed a LD block including two SNPs, rs2070746 and rs5030880, in both exudative AMD and PCV (Figure 1). However, haplotype-based association analysis could not identify any associated haplotype in exudative AMD or PCV (data not shown).

The heterozygous genotype of rs78488639 contributed a 2.05- and 2.27-fold of increased risk, respectively, to exudative AMD ($P = 0.043$) and PCV ($P = 0.016$; Table 2). Homozygous genotype of rs2070745 was associated with PCV ($P = 0.034$, OR = 1.80, 95% CI: 1.04–3.09). Heterozygous genotypes of rs2070746 and rs867229 were associated with a decreased risk in exudative AMD ($P = 0.019$, OR = 0.57, 95% CI: 0.35–0.91; $P = 0.0082$, OR = 0.54, 95% CI: 0.34–0.86, respectively). Heterozygous genotype of rs2070746 was also different between exudative AMD and PCV ($P = 0.0086$, OR = 0.51, 95% CI: 0.31–0.85). Moreover, five novel rare variants were identified. Amino-acid substitution prediction suggested that the protein function of c.368G > A (R123H) could be affected with a SIFT score of 0.01 (Table 3). This was further supported by the PolyPhen prediction with a score of 2.75. However, neither individual rare variant nor pooling of the variants were associated with exudative AMD or PCV (data not shown). The association became not significant after Bonferroni's correction ($P = 0.05/28 = 0.0018$).

The G allele of *CFH* rs800292 increased the risk for both exudative AMD (homozygous: OR = 2.60, 95% CI: 1.27–5.31, $P = 0.0074$; heterozygous: OR = 1.41, 95% CI: 0.68–2.92, $P = 0.36$) and PCV (homozygous: OR = 3.12, 95% CI: 1.42–6.86, $P = 0.0035$; heterozygous: OR = 2.50, 95% CI: 1.14–5.51, $P = 0.020$). The risk was also increased by the A allele of *HTRA1* rs11200638 in exudative AMD (homozygous: OR = 12.48, 95% CI: 6.53–23.83, $P = 1.07 \times 10^{-16}$; heterozygous: OR = 2.46, 95% CI: 1.31–4.64, $P = 0.0046$) and PCV (homozygous: OR = 5.24, 95% CI: 2.89–9.50, $P = 1.79 \times 10^{-8}$; heterozygous: OR = 2.51, 95% CI: 1.47–4.29, $P = 5.94 \times 10^{-4}$). Logistic regression analysis revealed that only *FPR1* rs78488639 remained significant after adjusting for gender and other individual-associated SNPs in both exudative AMD ($P = 0.032$) and PCV ($P = 0.022$). Further interaction analysis identified significantly positive interactions

Table 1 Sequence variants detected in *FPR1*

No.	Location	dbSNP ID	Sequence change	Codon change	Genotype frequency[a]			Genotype association P-value		Minor allele frequency (%)		
					AMD	PCV	Control	AMD vs Control	PCV vs Control	AMD	PCV	Control
1	Intron 1	rs7253284	c.1-514C>T	—	1/4/150	0/9/161	0/16/203	0.068	0.533	6 (1.9)	9 (2.6)	16 (3.7)
2	Intron 1	rs7253355	c.1-342C>T	—	11/54/90	14/68/88	5/85/129	0.070	0.020	76 (24.5)	96 (28.2)	95 (21.7)
3	Intron 1	Novel	c.1-55C>T	—	0/0/153	0/0/172	0/1/218	1.000	1.000	0 (0.0)	0 (0.0)	1 (0.2)
4	Intron 1	Novel	c.1-18A>T	—	0/0/153	0/2/170	0/0/219	—	0.193	0 (0.0)	2 (0.6)	0 (0.0)
5	Exon 2	rs5030878	c.32T>C	Ile11Thr	0/6/147	0/12/160	0/12/207	0.491	0.540	6 (2.0)	12 (3.5)	12 (2.7)
6	Exon 2	Novel	c.117C>T	Leu39Leu	0/17/136	2/14/156	0/18/201	0.347	0.278	17 (5.6)	18 (5.2)	18 (4.1)
7	Exon 2	Novel	c.279C>T	Phe93Phe	0/0/153	0/0/170	0/1/218	1.000	1.000	0 (0.0)	0 (0.0)	1 (0.2)
8	Exon 2	rs78488639	c.289C>A	Leu97Met	0/20/133	1/24/144	0/15/204	0.043	0.029	20 (6.5)	26 (7.7)	15 (3.4)
9	Exon 2	rs2070745	c.301G>C	Val101Leu	45/75/33	60/71/39	54/102/63	0.261	0.066	165 (53.9)	191(56.2)	210 (47.9)
10	Exon 2	rs28930680	c.306T>C	Phe102Phe	0/3/150	0/1/169	0/3/216	0.693	0.635	3 (0.7)	1 (0.3)	3 (0.7)
11	Exon 2	rs5030879	c.348C>T	Ile116Ile	0/2/151	0/2/168	0/1/218	0.571	0.583	2 (0.7)	2 (0.6)	1 (0.2)
12	Exon 2	Novel	c.368G>A	Arg123His	0/1/152	0/0/170	0/0/219	0.411	—	1 (0.3)	0 (0.0)	0 (0.0)
13	Exon 2	Novel	c.439A>T	Ile147Phe	0/0/155	0/2/173	0/2/217	0.513	1.000	0 (0.0)	2 (0.6)	2 (0.5)
14	Exon 2	Novel	c.513G>A	Thr171Thr	0/0/155	0/0/175	1/1/217	0.491	0.448	0 (0.0)	0 (0.0)	3 (0.7)
15	Exon 2	rs2070746	c.546C>A	Pro182Pro	37/60/58	33/95/47	52/108/59	0.065	0.462	134 (43.2)	161 (46)	212 (48.4)
16	Exon 2	Novel	c.553A>G	Asn185Asp	0/0/155	0/0/175	0/2/217	0.513	0.505	0 (0.0)	0 (0.0)	2 (0.5)
17	Exon 2	rs5030880	c.568A>T	Arg190Trp	4/46/105	4/63/108	6/76/137	0.580	0.934	54 (17.4)	81 (20.3)	88 (20.1)
18	Exon 2	rs1042229	c.576T>G[b]	Asn192Lys	—	—	—	—	—	—	—	—
—	—	—	c.576T>C[b]	Asn192Asn	—	—	—	—	—	—	—	—
19	Exon 2	Novel	c.721C>T	Arg241Trp	0/0/155	0/0/175	0/1/218	1.000	1.000	0 (0.0)	0 (0.0)	1 (0.2)
20	Exon 2	Novel	c.944 G >A	Arg309Gln	0/0/153	0/2/176	0/0/220	—	0.199	0 (0.0)	2 (0.6)	0 (0.0)
21	Exon 2	rs867228	c.1037C>A	Glu346Ala	17/63/73	16/81/81	17/108/95	0.249	0.750	97 (31.7)	113 (31.7)	142 (32.3)
22	3′-UTR	Novel	c.1053+8G>T	—	0/0/153	0/0/178	0/1/219	1.000	1.000	0 (0.0)	0 (0.0)	1 (0.2)
23	3′-UTR	rs867341	c.1053+75A>G	—	1/7/145	0/5/173	0/7/213	0.378	0.829	9 (2.9)	7 (1.4)	7 (1.6)
24	3′-UTR	Novel	c.1053+109G>C	—	0/1/152	0/0/178	0/0/220	0.410	—	5 (1.6)	0 (0.0)	0 (0.0)
25	3′-UTR	Novel	c.1053+162T>G	—	0/0/152	0/1/170	0/0/220	—	0.437	0 (0.0)	1 (0.3)	0 (0.0)
26	Downstream	rs867229	c.1053+196C>T	—	29/50/75	18/79/74	23/107/87	0.0026	0.808	108 (35.1)	115 (33.6)	153 (35.2)
27	Downstream	rs1868943	c.1053+205C>T	—	0/2/152	0/0/171	0/8/209	0.205	0.010	2 (0.7)	0 (0.0)	8 (1.8)
28	Downstream	Novel	c.1053+219G>A	—	0/1/153	0/3/168	0/1/216	1.000	0.325	1 (0.3)	3 (0.9)	1 (0.2)

[a] Genotype frequency presented as number of individual with homozygote/heterozygote/wild-type genotypes
[b] Genotype and allele frequencies of c.576T>G/C is presented in a separate table.

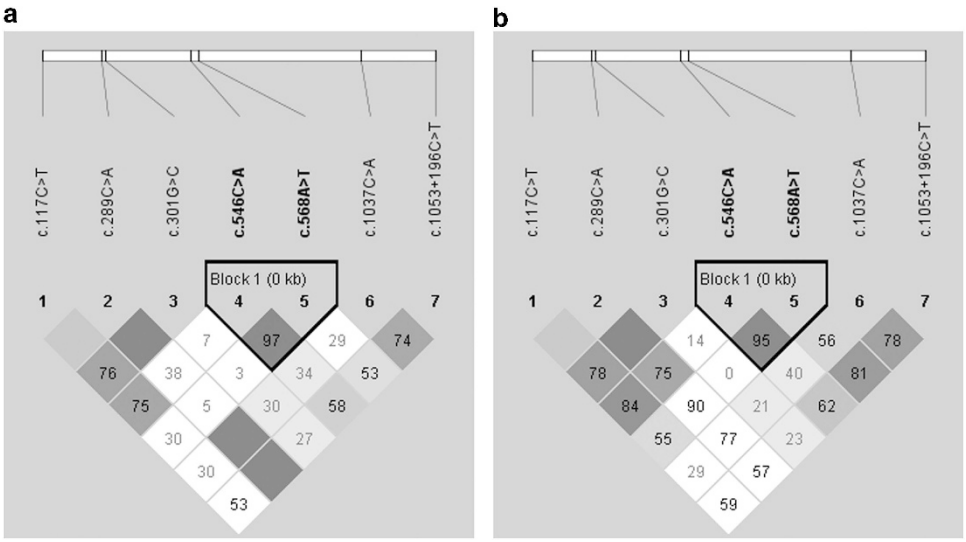

Figure 1 Haplotype block structure for the seven common polymorphisms. The haplotype analysis revealed a LD block lying across rs2070746 and rs5030880 in (a) AMD and (b) PCV.

among *FPR1* rs78488639, *CFH* rs800292, and *HTRA1* rs11200638 in exudative AMD ($P = 0.022$) and PCV ($P = 0.023$). In the combined effect analysis, ORs of combined *FPR1* rs78488639 CA and *CFH* rs800292 GG genotypes were approximately twice greater than the individual ORs of *FPR1* rs78488639 CA or *CFH* rs800292 GG in both exudative AMD ($P = 0.0062$, OR = 4.83, 95% CI: 1.51–15.51) and PCV ($P = 0.019$, OR = 4.03, 95% CI: 1.22–13.28; Table 4). A combined risk OR of 10.47 ($P = 2.22 \times 10^{-4}$, 95% CI: 2.72–40.29) was identified in the heterozygous risk alleles of these two variants in the PCV patients, but not in exudative AMD ($P = 0.133$). In addition, ORs of combined *FPR1* rs78488639 CA and *HTRA1* rs11200638 AA genotypes were at least 1.5-fold higher than the individual ORs of *HTRA1* rs11200638 AA and >6-fold higher than *FPR1* rs78488639 CA in both exudative AMD ($P = 1.02 \times 10^{-4}$, OR = 19.47, 95% CI: 3.75–100.97) and PCV ($P = 7.45 \times 10^{-4}$, OR = 14.19, 95% CI: 2.72–74.20). Furthermore, combined ORs of *FPR1* rs78488639 CA and smoking were >5-fold higher than *FPR1* rs78488639 CA in both exudative AMD ($P = 0.010$, OR = 10.93, 95% CI: 1.30–92.10) and PCV ($P = 9.96 \times 10^{-4}$, OR = 16.94, 95% CI: 2.06–139.38; Table 5).

Discussion

Inflammation is an important component in AMD etiology. Chronic localized inflammation initiated and stimulated by drusen, together with the accumulation of lipofuscin, compromise RPE function.[28,29] The injured RPE actively recruits choroidal dendritic cells,[29] microglia, macrophages,[30] and leukocytes,[31] which express VEGF and stimulate CNV growth,[32] the hallmark of exudative AMD. The initiation of AMD by inflammation and infections has been supported by clinical observations of *Chlamydia pneumonia* and cytomegalovirus infections in AMD patients.[33,34] As FPR1 is a receptor for phagocytic cells recognizing the presence of exogenous organisms and mediating the traffic of phagocytes to the sites of tissue damage,[18] FPR1 could have an important role in AMD initiation by infections.

The involvement of *FPR1* in innate immunity is supported by multiple evidences. First, the release of formylated peptides from mitochondria secondary to cell death might attract phagocytic leukocytes through the receptor.[35] Second, the *Fpr1*$^{-/-}$ mice model had accelerated mortality and increased bacterial burden in the liver and spleen early after infection,[36] showing a role of *Fpr1* in the host defense. Moreover, rs5030879 (c.348C>T, I116I) in *FPR1* is associated with aggressive periodontitis,[37] which is an infectious–inflammatory disease. In addition, c.329T>C (F110S), c.378C>G (C126W), and rs5030878 (c.32C>T, I11T) of *FPR1* are significantly correlated with defective polymorphonuclear neutrophil (PMN) function and C-reactive protein.[38,39] However, limited data reported the association of *FPR1* with AMD, and also PCV. In this study, the results of *FPR1* sequencing in both exudative AMD and PCV revealed five associated polymorphisms (Table 1), indicating a role of *FPR1* in the inflammation mechanisms of exudative AMD and PCV.

Formyl peptide receptors (FPRs) are expressed mainly in PMNs and monocytes, but also found in neurons, microglial, and glial cells.[40] In the inflammatory

Table 2 Odds ratios of *FPR1*-associated polymorphisms

SNP ID	*Sequence change*	*Homo vs WT*					*Hetro vs WT*				
		AMD–Control	*OR (95% CI)*	*PCV–Control*	*OR (95% CI)*	*AMD–PCV*	*AMD–Control*	*OR (95% CI)*	*PCV–Control*	*OR (95% CI)*	*AMD–PCV*	*OR (95% CI)*
rs78488639	c.289C>A	—	—	0.415	1.01 (0.99–1.02)	1.000	0.043	2.05 (1.01–4.14)	0.016	2.27 (1.15–4.47)	0.752	0.90 (0.48 –1.71)
rs2070745	c.301G>C	0.114	1.59 (0.89–2.84)	0.034	1.80 (1.04–3.09)	0.695	0.197	1.40 (0.84–2.35)	0.646	1.12 (0.68–1.86)	0.442	1.25 (0.71–2.20)
rs2070746	c.546C>A	0.254	0.72 (0.42–1.26)	0.443	0.80 (0.45–1.42)	0.757	0.019	0.57 (0.35–0.91)	0.681	1.10 (0.69–1.77)	0.0086	0.51 (0.31–0.85)
rs867229	c.1053+196C>T	0.234	1.46 (0.78–2.74)	0.813	0.92 (0.46–1.84)	0.173	0.0082	0.54 (0.34–0.86)	0.514	0.87 (0.57–1.33)	0.053	0.62 (0.39–1.0)

Note: There was no homozygous genotype of rs78488639 identified in AMD and control group.

response, phagocytes are induced to move directionally through chemotaxis.[41] When the ligands (N-formyl peptides) bind to *FPR1*, *FPR1* will direct the activated phagocyte migration through the PI3k/PTEN pathways. This implies an important role of *FPR1* in inflammatory cell migration. *FPR1* also induces superoxide production, and this oxidative stress could further damage the RPE cells.[42,43] Furthermore, *FPR1* expression can be enhanced by inflammation in Alzheimer's disease,[44] which is associated with age as AMD and involves accumulation of amyloid β (Aβ) in senile plaques. Aβ has been found in AMD drusen.[45] Therefore, FPR1 likely has an etiological role in AMD.

CFH and *HTRA1* are well recognized as susceptibility genes for AMD.[22,23] *CFH* is an inhibitor in alternative complement pathway, controlling complement activation.[46] Abnormal regulation of complement activation by *CFH* might contribute to AMD pathogenesis.[47] HTRA1 is also involved in inflammatory responses.[48,49] In this study, *FPR1* significantly interacted with *CFH* and *HTRA1*, with respective combined ORs of 10.47 and 19.47 for increasing disease risk (Table 4). Moreover, the risks of *FPR1* to exudative AMD and PCV were also increased by smoking, with respective combined ORs of 10.93 and 16.94 (Table 5). The combined effect with smoking has been demonstrated in our previous studies on *CFH* and *HTRA1*.[22,23] Therefore, the role of *FPR1* as well as the contribution of inflammation genes and smoking to AMD and PCV are further implicated.

SNP rs1042229 is a triallelic (T, G or C) variant, whereas the majority of SNPs are biallelic. Its G allele specifies lysine and T/C specifies asparagines at codon 192. The N192 locates in the center of the second extracellular loop, which the lysine substitution may alter the ligand-binding specificity and binding affinity through the binding pocket for N-formyl peptides.[50] In this study, the G allele is the risk allele of rs1042229, and homozygous G genotype of rs1042229 was associated with 2.27-fold of increasing risk in exudative AMD, but not in PCV. Another variant (L97M; rs78488639), located in the first extracellular loop, markedly decreases in the affinity of ligand binding.[51] This variant showed significant association with both exudative AMD and PCV, contributing an approximately twofold increasing disease risk (Table 2). Differential associations of various *FPR1* SNPs with AMD and PCV are also noted, suggesting that exudative AMD and PCV might have different gene variant association patterns.

In summary, significant interactions and combined effects of *FPR1* with *CFH*, *HTRA1*, and smoking in exudative AMD and PCV were revealed in this study. *FPR1* could have a role in AMD and PCV pathogenesis, and its biological functions in the disease mechanisms need to be elucidated.

Table 3 Amino-acid substitution prediction of novel rare *FPR1* variants

Sequence change	*Codon change*	*Genotypic frequency*			*Predictions*			
		AMD	*PCV*	*Control*	*SIFT*	*Score*	*Polyphen*	*Score*
c.368G>A	R123H	0/1/152	0/0/170	0/0/219	Affect protein function	0.01	Probably damaging	2.753
c.439A>T	I147F	0/0/155	0/2/173	0/2/217	Tolerated	0.29	Benign	0
c.553A>G	N185D	0/0/155	0/0/175	0/2/217	Tolerated	0.69	Benign	0.525
c.721C>T	R241W	0/0/155	0/0/175	0/1/218	Tolerated	0.09	Benign	0.807
c.944G >A	R309Q	0/0/153	0/2/176	0/0/220	Tolerated	0.22	Benign	0.538

Table 4 Combined effects of *FPR1* rs78488639 with *CFH* rs800292 and *HTRA1* rs11200638 in exudative AMD and PCV

		CFH rs800292					
		AA		*GA*		*GG*	
	FPR1 rs78488639 OR_{FPR1}*(95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*
Exudative AMD							
CC	1.00 (Ref)		1.00 (Ref)	0.317	1.52 (0.66–3.49)	0.0083	2.90 (1.29–6.54)
CA	2.05 (1.01–4.14)	0.321	3.22 (0.55–18.85)	0.196	3.22 (0.67–15.56)	0.0062	4.83 (1.51–15.51)
PCV							
CC	1.00 (Ref)	—	1.00 (Ref)	0.090	2.01 (0.89–4.55)	0.020	2.58 (1.14–5.84)
CA	2.27 (1.15–4.47)	1.000	0.91 (0.81–1.01)	2.22×10^{-4}	10.47 (2.72–40.29)	0.019	4.03 (1.22–13.28)
AA	1.01 (0.99–1.02)	—	—	—	—	0.256	1.11 (0.90–1.37)

		HTRA1 rs11200638					
		GG		*GA*		*AA*	
	FPR1 rs78488639 OR_{FPR1}*(95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*
Exudative AMD							
CC	1.00 (Ref)	—	1.00 (Ref)	0.083	1.81 (0.82–3.57)	1.94×10^{-13}	11.02 (5.55–21.90)
CA	2.05 (1.01–4.14)	1.000	0.70 (0.08–6.08)	3.83×10^{-4}	8.11 (2.56–25.74)	1.02×10^{-4}	19.47 (3.75–100.97)
PCV							
CC	1.00 (Ref)	—	1.00 (Ref)	0.0018	2.59 (1.41–4.76)	3.15×10^{-8}	6.20 (3.16–12.16)
CA	2.27 (1.15–4.47)	0.037	4.06 (1.26–13.04)	0.0023	6.08 (1.92–19.30)	7.45×10^{-4}	14.19 (2.72–74.20)
AA	1.01 (0.99–1.02)	—	—	—	—	0.207	1.06 (0.95–1.17)

Note: Allele A is the risk allele for *FPR1* rs78488639, allele G is risk allele for *CFH* rs800292, and allele A is the risk allele for *HTRA1* rs11200638.

Table 5 Combined effects of *FPR1* rs78488639 with smoking in exudative AMD and PCV

		Smoking			
		No		*Yes*	
	FPR1 rs78488639 OR_{FPR1}*(95% CI)*	P	*OR (95% CI)*	P	*OR (95% CI)*
Exudative AMD					
CC	1.00 (Ref)	—	1.00 (Ref)	0.038	1.93 (1.04–3.58)
CA	2.05 (1.01–4.14)	0.739	0.67 (0.16–2.74)	0.010	10.93 (1.30–92.10)
PCV					
CC	1.00 (Ref)	—	1.00 (Ref)	0.992	1.00 (0.48–2.10)
CA	2.27 (1.15–4.47)	0.164	2.15 (0.72–6.44)	9.96×10^{-4}	16.94 (2.06–139.38)
AA	1.01 (0.99–1.02)	—	—	—	—

Note: Allele A is the risk allele for *FPR1* rs78488639.

Summary

What was known before

- Inflammation has an influential role in exudative AMD. Inflammation-related genes should be studied in AMD patients. The *FPR1* gene is involved in inflammatory responses by the activation of chemotatic peptides. *FPR1* is a crucial molecule in innate immunity. However, the role of *FPR1* in AMD pathogenesis has yet to be studied.

What this study adds

- This genetic study discovered the association of *FPR1* gene with AMD and PCV. In addition, *FPR1* gene also significantly interacts with *CFH* and *HTRA1* genes as well as smoking in both exudative AMD and PCV.

Conflict of interest

The authors declare no conflict of interest.

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