rs3753394 Complement Factor H (CFH) Gene Polymorphism in Patients with Age-Related Macular Degeneration (AMD) in Indonesian Population

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Abstract. Neovascular Age-related Macular Degeneration (nAMD) is one of the major factors for blindness and impaired visual acuity in elderly people. The aim of this study was CFH gene screening in Age-Related Macular Degeneration patients in Indonesia. This study was performed in 106 AMD patients and 104 controls for genomic markers in the Complement Factor H (*CFH*). The diagnosis of AMD was carried out by retinal specialists based on color fundus photography and optical coherence tomography. Informed consent was given to patients then proceed to blood sampling and recording of body parameters (BMI, smoking, other systemic diseases). *CFH* polymorphisms were then analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP). There was no association between genetics polymorphism with nAMD. From the research can be inferred that association between genetics polymorphism with nAMD was insignificant.

1 Introduction

Neovascular Age-related macular degeneration (nAMD) was the serious risk factor in elderly people central sight loss in developed countries [1], [2] Approximately 200 millions people had AMD in 2020. The number was predicted to be increased to 288 millions in 2040. AMD's risk is primarily caused by advanced age and was also influenced by DNA mutations as well as lifestyle.[3]–[5]

Polymorphism in complement gene family, in particular, were closely related to the AMD epidemiology and biology.[4], [6]–[15]. Complement was essentials part of innate immune system which involved in opsonizing, removing bacteria and death cell degradation. Complement factor also involved in immune cells recognition to infection and tissue injury sites.[16], [17].

The significance factors of complement protein in AMD onset remains uncertain. In case lampalizumab, a factor D inhibitor which inhibit of the alternative complement pathway activation, was fail in a phase III clinical trial. Lampalizumab had no effect on the halting progression of drusen enlargement in severe dry AMD patients [18].

The genetic correlations in AMD have been extensively documented in the literature[10], [12], [19]. The Y402H mutation in complement factor H (CFH) [20]–[24] was one of the most widely studied and replicated genetic variations linked to AMD risk.

In Western populations, CFH connections were found in Dutch, American, Italian, Spanish, and Swiss people are a few examples. Similar relationships have been seen in Asian populations such Japanese[25], Chinese[26], [27], and Indians[28]. However, evidence from Asia's Malay community, which is one of Asia's major ethnic groups, is few. An earlier study in Indonesia found a link between Y402H and AMD [20].

The aim of this study was to look at the relationship between rs3753394 CFH and AMD in the Indonesian population.

2 Methods

2.1 Patient recruitment and data collection

The research procedure related to patients was approved by the Ethics Committee (Ref. No.: KE/FK/021/EC/2021). During patient and control screening, 106 eligible AMD and 104 age-matched subjects were recruited. Patient and control were screened from January to August 2019. All participants were understood and signed the informed consent before ophthalmic test and blood collection. To avoid interference, the authors only recruit patients who have no additional retinal or systemic disorders. To diagnose nAMD or confirm the control eye group, all patients receive a complete ophthalmic test that includes BCVA, fundoscopy, and optical coherence tomography (OCT).

This investigation employed spectral-domain OCT in separating nAMD from polypoidal choroidal vasculopathy (PCV). When compared to ICGA, the diagnosis of nAMD exceeded 90% matching data, indicating that there was minimal bias in this investigation.

A systematic questionnaire was utilized to assessed AMD and control patients' lifestyles, smoking status, and working activity.

2.2 Genotyping

Each patient's DNA was isolated from venous blood which stored in vacotainer containing EDTA. In this research, DNA extraction kit (GeneAid Genomic Human DNA Mini Kit [GB100/300], New Taipei City, Taiwan) was used to process the blood samples.

The specific variants rs3753394 was amplificated using thermal cycler. The machine was set as 1 cycle (95 °C for 10 min), 30 cycles (95 °C for 30 s; 55 °C for 60 s; 72 °C for 60 s), and 1 cycle (72 °C for 5 min). The primer used in this research was F: 5'-CACAATAGACCCGAATAGAGT-3' and R: 5'-GAAATGCCAGAAGTTAAACC-3'[29].

The PCR product was digested using EcoRV for genotype determination (Figure 1.). The PCR product was incubated at 37 °C for 18 h. The digested PCR products were run on 2% agarose gel.
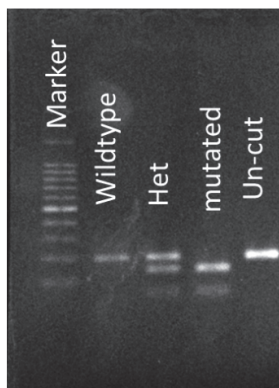

Figure 1. The digested PCR product visualization using agarose gel electrophoresis.

2.3 Statistical analysis

Data analysis was done using Crosstabs (Chi-square tests) and logistic regression models measured by odds ratio (OR) and 95% confidence interval (CI). All analyses were carried out using SPSS (version 25, IBM Corp).

3 Results

An ophthalmologist made the diagnosis of all AMD or age-matched controls. 104 nAMD patients [46 males (44.2%) and 58 females (55.8%)] and 100 age-matched controls [45 males (45.0%) and 55 females (55.0%)] data were included for statistical analysis, shown in Table 1.

Table 1. Baseline characteristics of nAMD and age-matched control

	nAMD (n=104)	**Control (n=100)**	**P**
Gender Male Female	46 (44.2%) 58 (55.8%)	45 (45.0%) 55 (55.0%)	0.91
Age, year 40-49 50-59 60-69 70-79 >80	6 (5.8%) 20 (19.2%) 35 (33.7%) 39 (37.5%) 4 (3.8%)	1 (1.0%) 7 (7.0%) 50 (50.0%) 38 (38.0%) 4 (4.0%)	0.16
Race Javanese Non-Javanese	97 (93.3%) 7 (6.7%)	97 (97.0%) 3 (3.0%)	0.18
Genotype GG GT TT	33 (31.7%) 50 (48.1%) 21 (20.2%)	27 (27.0%) 50 (50.0%) 23 (23.0%)	0.78

Table 2. Genotype Association and Odds Ratio for CFH Genotype.

Genotype	Genotype Distribution		Genotype Association (p)	OR	95% CI
	nAMD (n=104)	Control (n=100)			
GT	33 (55%)	27 (45%)	0.540	0.82	0.43-1.56
TT	50 (50%)	50 (50%)	0.802	1.095	0.54-2.23
GG	21 (47.7%)	23 (52.3%)	–	1.00	Ref

Table 2 summarizes the genotype distributions, genotype associations, odds ratio (OR), and 95% confidence interval of CFH Y402H. The GG genotype is used as indicator. It is worth noting that the TT genotype is the most prevalent in both groups, whereas the GG genotype is the least common. Both GT and TT genotypes have p>0.05 indicates that they were not significantly associated.

4 Discussions

The findings of this investigation revealed that CFH polymorphisms were not significantly linked to AMD.

rs3753394 was not associated with nAMD in Indonesia. Our results similar to Japan[30], Spanish[31], but not to Korea[32] Caucasian[12] and China[26], [33] population. These results indicated that this SNP is not specific and cannot be determined as marker in Malay race.

rs3753394 mutation occur in the promoter region of *CFH* which regulate the gene expression. In other epidemiological study, rs3753394 is among the strong SNPs beside rs1061170 (Y402H), rs10737680 and rs800292 (I62V) within CFH gene.

Complement Factor H (*CFH*) was the most associated genes beside *ARMS2* and *HTRA1* in nAMD. Despite the fact that additional variables may play a role in AMD pathogenesis, complement regulatory pathways could be viable therapeutic targets in nAMD.

Our study's strengths are the age-matched participant, extensive ophthalmic examinations by vitreo-retina specialists employing modern multimodal imaging to confirm the diagnosis of nAMD. Genotyping was utilized PCR-RFLP to assure genetic assessment accuracy.

Another drawback of our study is that because it was conducted in a hospital, the advanced profile of AMD patients may have been captured, and so the representation of AMD in the general community is limited. It's still unclear whether people with AMD in the broader community have comparable genetic correlations. To answer these problems, further population-based research is needed.

5 Conclusion

The rs3753394 CFH gene polymorphism was neither statistically or clinically significant in persons with AMD in Yogyakarta, Indonesia, according to this study. We are aware that the patient group's short sample size may have resulted in poorer statistical power, and that a larger and more diversified sample size would have been preferable to allow sub-analysis based on Indonesia's ethnic origin for future studies.

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