

# Analysis of the association between *CFH* Y402H polymorphism and response to intravitreal ranibizumab in patients with neovascular age-related macular degeneration (nAMD)

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## ABSTRACT

Pharmacogenetic studies indicate that a variable response to anti-vascular endothelial growth factor (VEGF) therapy in patients with neovascular form of AMD (nAMD) may be due to polymorphisms in the complement factor H gene (*CFH*). This study is the first to investigate the association between *CFHY*402H polymorphism and the response to ranibizumab therapy in Malaysian patients with nAMD. We included 134 patients with nAMD, examined between September 2014 and February 2016. The diagnosis of nAMD was confirmed by ophthalmologic examination, before ranibizumab therapy was started. Each patient received an intravitreal injection of 0.5 mg/0.05 ml ranibizumab following a treat-and-extend (TE) regimen. Best-corrected visual acuity (BCVA) and central retinal thickness (CRT) were recorded after 3 and 6 months following the first injection and compared with the baseline values. Genotyping of Y402H (rs1061170) polymorphism was performed using PCR-RFLP and the amplified product was digested with MluCI restriction enzyme. Association between the Y402H genotypes and response to treatment was determined by a logistic regression analysis of responder (n = 49) and non-responder (n = 84) group. Significantly worse mean BCVA was observed for the CC genotype compared to the TT + CT genotype in the total sample after 6-month follow-up ( $p = 0.018$ ). Comparing the baseline and 6-month point measurements, improved mean BCVA was observed in responder group, while worse mean BCVA was recorded for non-responder group. However, our regression analysis, adjusted for confounding factors, showed no significant association between the Y402H genotypes and response to treatment in nAMD patients under the recessive model ( $p > 0.05$ ). Overall, our results suggest that factors other than Y402H polymorphism may be involved in the progression of nAMD after treatment with anti-VEGF agents, in Malaysian population.

KEY WORDS: Age-related macular degeneration; AMD; complement factor H; CFH; ranibizumab; Y402H; SNP; treatment response

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## INTRODUCTION

In developed countries, especially in the West, age-related macular degeneration (AMD) is the major cause of visual

impairment in older adults [1-3]. Today, a similar trend is observed in a large number of Asian countries, where pharmacogenetic studies on AMD have shown unique epidemiological, genetic, and clinical features of AMD in Asian populations, as well as a different response to treatment, compared to the populations in the West. This may be related to several factors, such as a higher number of different racial and ethnic groups in Asia, the use of a different classification system for AMD, and dietary and lifestyle factors [4]. The neovascular form of AMD (nAMD), associated with choroidal

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neovascularization (CNV), is a vasculopathy resulting in serous exudation and hemorrhages [5]. Angiogenesis and vascular permeability during the progression of CNV are regulated by vascular endothelial growth factor (VEGF), among other factors. Bevacizumab [6] and ranibizumab [7] are anti-VEGF drugs commonly used to improve best-corrected visual acuity (BCVA) in AMD patients [8], and clinical studies in different populations demonstrated the efficacy of ranibizumab in treating nAMD [6,7,9].

Both environmental and genetic factors play a role in the development of nAMD [10]. The complement system of plasma proteins, important in the immune and inflammatory response of the body, is also involved in AMD pathogenesis. Complement factor H (CFH) is a regulator of the alternative pathway of the complement system; it has anti-inflammatory effects and ensures that host tissue is not damaged. Genetic variation in the *CFH* gene, which is located on 1q31.3 region of chromosome 1, has been associated with an increased risk of inflammatory diseases [11]. The T1277C polymorphism (rs1061170) of the *CFH* gene, involving a T to C transition at the 1277 position in the exon 9, and resulting in a substitution of tyrosine with histidine at the 402 position (Y402H) in the amino acid sequence, has been extensively studied in relation to the development of nAMD [12-16]. A number of pharmacogenetic studies in different countries, focused on the response of nAMD patients to anti-VEGF therapy [6-9,11,17-21], and indicated that a variable response to ranibizumab or bevacizumab therapy in nAMD patients may be due to polymorphisms in the *CFH* gene, among other factors. For example, the CC genotype of *CFHY402H* polymorphism has been associated with a poor response while the TT genotype with a good response to intravitreal injection of ranibizumab or bevacizumab [11,17-20].

To the best of our knowledge, such results have not been reported for the Malaysian population. In this study, we investigated the association between *CFH* Y402H polymorphism and the response to ranibizumab therapy in Malaysian patients with nAMD.

## MATERIALS AND METHODS

The study was approved by the Universiti Putra Malaysia (UPM), Universiti Kebangsaan Malaysia (UKM) Ethics Committee and Medical Research Ethics Committee (MREC), as well as by the Ministry of Health (MOH) in Malaysia, and registered in the National Medical Research Registry with a registration number NMRR-14-1176-21475.

### Data collection

This prospective cohort study was conducted from September 2014 to February 2016 at the ophthalmology clinics

of two tertiary centers, i.e., Hospital Selayang and Universiti Kebangsaan Malaysia Medical Centre (UKMMC). Out of 158 patients with nAMD that were invited to participate in the study, 134 patients were included. Informed consent was obtained from all participants before initiating the study.

### Patient's criteria

Before therapy with ranibizumab was started, comprehensive ophthalmologic examination was performed in all patients and the diagnosis of nAMD was made based on BCVA test and Snellen chart, slit-lamp examination, dilated fundus examination, fluorescein angiography (FA), measurement of central retinal thickness (CRT) using optical coherence tomography (OCT) and indocyanine green angiography (ICG). Active neovascularization was confirmed with FA and OCT at baseline, before treatment. None of the patients were previously treated for nAMD.

All patients aged above 50 years, with the presence of exudative or hemorrhagic features involving macula, with no previous intravitreal anti-VEGF treatment, and with minimum follow-up of 6 months after the first injection of ranibizumab (Lucentis; Novartis Pharma AG, Basel, Switzerland and Genentech Inc., South San Francisco, California, USA) were eligible for this study.

Patients who were followed up for less than 6 months, had a CNV secondary to other causes such as trauma, hereditary diseases, angioid streaks, inflammatory disease, pathologic myopia, had undergone a posterior vitrectomy, or had retinal diseases other than AMD were excluded from this study.

### Measures of response to treatment

Each patient received an intravitreal injection of 0.5 mg/0.05 ml ranibizumab for the first 3 months. After the initial 3 months, if leakage was still present, the patients were receiving a monthly injection until the leakage stopped. Once the leakage was under control, a treat-and-extend (TE) regimen was applied by extending the treatment interval for a few weeks, based on the clinical examination and presence of sub-retinal fluid on OCT or leakage on FA. If the disease remained stable at each visit, the treatment interval was extended for another 1 to 2 weeks, however, if there was recurrence of leakage, the treatment with a monthly injection was continued until the leakage stopped. The patient's medical history, including cigarette smoking, presence of diabetes and hypertension and symptoms of nAMD, was recorded. All patients were scheduled for follow-up, and BCVA and CRT were recorded after 3 and 6 months following the first injection of ranibizumab and compared with the baseline values. Based on the changes in BCVA and CRT, the patients were classified as either responders or non-responders. The responder group was defined as patients having an improvement of three lines or higher in the

Snellen chart and/or having a decrease of more than 100  $\mu\text{M}$  in CRT after 6 months following the first ranibizumab injection, compared to the baseline measurement. The non-responder group was defined as patients having no changes in BCVA and CRT or having a reduction of three lines or more in the Snellen chart and/or an increase of 100  $\mu\text{M}$  in CRT after 6 months of first injection, compared to the baseline. A 2-ml blood sample was collected from each patient and transferred to blood collection tubes containing EDTA, for genotype analysis.

## Genotyping

The genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and stored at  $-20^{\circ}\text{C}$  for further analysis. The genotyping of CFHY402H (rs1061170) polymorphism was performed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The PCR amplification was performed in a thermocycler (Thermo Fisher Scientific, Waltham, MA USA) with the following conditions:  $95^{\circ}\text{C}$  for 5 minutes as initial denaturation, followed by 35 cycles (denaturation at  $95^{\circ}\text{C}$  for 15 seconds, annealing of forward primer [5'-TCATTGTTATGGTCCTTAGGAAA-3'] and reverse primer [5'-GGAGTAGGAGACCAGCCATT-3'] at  $57^{\circ}\text{C}$  for 15 seconds, and extension at  $72^{\circ}\text{C}$  for 45 seconds). The final cycle was extension at  $72^{\circ}\text{C}$  for 5 minutes.

The amplified product, a 179-bp fragment, was then digested with MluCI restriction enzyme (New England Biolabs, Beverly, MA, USA) at  $65^{\circ}\text{C}$  for 3 hours. The digested products were separated on agarose gel (2%) and visualized using a UV Alpha imager (Alpha Innotech, San Leandro, CA, USA). Random samples were used to validate the results, and identical results were obtained.

## Statistical analysis

BCVA determined by the Snellen chart was converted into a logarithm of minimal angle of resolution (logMAR) for further statistical analysis. The descriptive statistics for all demographic and clinical variables were compared using the Student's *t*-test and one-way analysis of variance (ANOVA) for normal continuous variables, Mann-Whitney *U* test for

skewed continuous variables and Chi-square test for categorical variables. A logistic regression analysis was performed to determine the association between the CFHY402H genotypes and response to ranibizumab treatment. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA), and  $p < 0.05$  was considered statistically significant.

## RESULTS

### Patient characteristics, and mean baseline BCVA and CRT in relation to CFHY402H genotypes

The CFHY gene sequence was first amplified with PCR, the resulting 241-bp PCR product was then digested with MluCI restriction enzyme, and the following fragments for CFHY402H were obtained: 119-bp fragment for TT wild-type genotype, 179-bp and 119-bp fragments for CT heterozygous genotype, and 179-bp fragment for CC homozygous mutant genotype. We compared the baseline characteristics of patients and the mean baseline BCVA and CRT between the CFHY402H genotypes.

The baseline characteristics of patients are presented in Table 1. Out of 134 patients with nAMD, 126 (94.0%) had TT or CT genotype of CFHY402H, while 8 (6.0%) had CC genotype. A significant difference was observed in the distribution of CFHY402H genotypes in relation to the gender ( $p = 0.004$ ); i.e. a higher number of males had TT+CT genotypes ( $n = 81$  for males,  $n = 45$  for females) while a higher number females had CC genotype ( $n = 7$  for females,  $n = 1$  for males). The distribution of the CFHY402H genotypes was also significantly different with regard to the mean age; patients with the TT+CT genotypes had a higher mean age ( $69.76 \pm 7.56$  years) compared to those with the CC genotype ( $63.75 \pm 7.15$ ) [ $p = 0.030$ ]. There was no significant difference in the mean baseline BCVA and CRT between the CFHY402H genotypes ( $p > 0.05$ ).

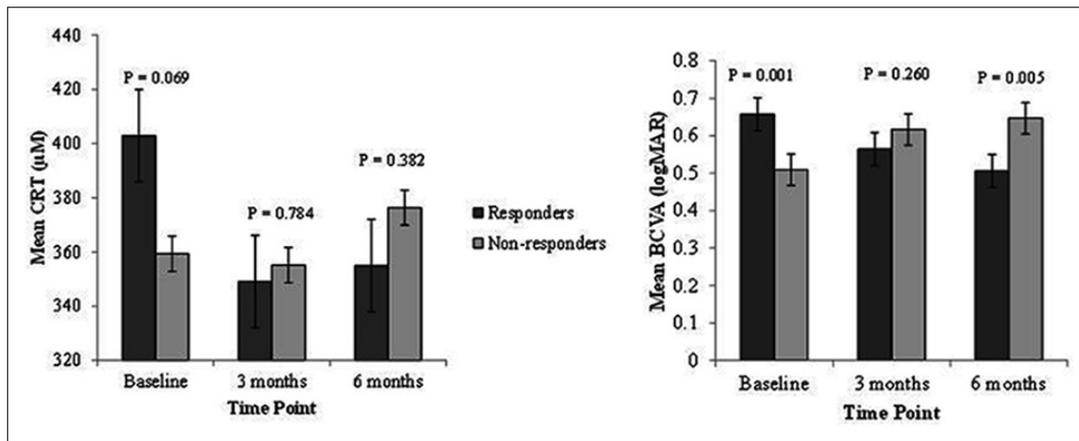
### Changes in BCVA and CRT after ranibizumab treatment in relation to CFHY402H genotypes

The mean BCVA and CRT obtained at baseline and after 3 and 6 months of first intravitreal injection of ranibizumab were compared between the CFHY402H genotypes. In addition,

**TABLE 1.** Baseline characteristics of study population

Parameter	TT+CT	CC	Total	<i>p</i> <sup>c</sup>
Number of patients <sup>b</sup>	126 (94.0)	8 (6.0)	134 (100)	
Mean age (years) <sup>a</sup>	69.76 $\pm$ 7.56	63.75 $\pm$ 7.15	69.40 $\pm$ 7.64	0.030 <sup>c</sup>
Gender (n)				
Male	81	1	82	0.004 <sup>d,e</sup>
Female	45	7	52	
Mean BCVA (logMAR) <sup>a</sup>	0.56 $\pm$ 0.26	0.66 $\pm$ 0.25	0.56 $\pm$ 0.26	0.262 <sup>c</sup>
Mean CRT ( $\mu\text{M}$ ) <sup>a</sup>	379.8 $\pm$ 136.42	308.5 $\pm$ 63.36	375.6 $\pm$ 134.1	0.145

<sup>a</sup>Data are presented as mean $\pm$ standard deviation (SD). <sup>b</sup>Data are reported as number (percentage) of patients. <sup>c</sup>Student's *t*-test. <sup>d</sup>Chi-squared test. <sup>e</sup>Mann-Whitney *U* test. \*Significant *p* value,  $p < 0.05$ . CRT: Central retinal thickness; BCVA: Best-corrected visual acuity



**FIGURE 1.** Patients with nAMD (N = 134) were classified into responder (n = 49) and non-responder group (n = 84). The mean CRT was not significantly different between the two groups ( $p > 0.05$ ), but the mean BCVA at baseline ( $p = 0.001$ ) and after 6 months of the first injection ( $p = 0.005$ ) was significantly different between the two groups. At baseline, worse BCVA was observed for responder ( $0.66 \pm 0.26$  logMAR) compared to non-responder group ( $0.51 \pm 0.25$  logMAR). However, after 6 months of first injection, improved mean BCVA was observed in responder group ( $0.51 \pm 0.26$  logMAR) compared to the corresponding baseline measurement, while the mean BCVA in non-responder group was worse after 6 months ( $0.65 \pm 0.29$  logMAR) [ $p = 0.005$ ]. nAMD: Neovascular age-related macular degeneration; CRT: Central retinal thickness; BCVA: Best-corrected visual acuity.

**TABLE 2.** Visual acuity and central retinal thickness in relation to the *CFH* Y402H genotypes in patients with nAMD

Parameter	Time point	TT+CT	CC	$p^b$
Mean BCVA (logMAR) <sup>a</sup>	Baseline	0.56±0.26	0.66±0.25	0.262
	3 months	0.59±0.26	0.70±1.93	0.185
	6 months	0.58±0.28	0.84±0.24	0.018*
Mean CRT (µM) <sup>a</sup>	Baseline	379.8±136.42	308.5±63.36	0.145 <sup>c</sup>
	3 months	352.7±124.58	356.8±92.51	0.707
	6 months	363.7±134.63	442.3±154.6	0.115
Changes in BCVA (logMAR) <sup>a</sup>	3 months	0.03±0.22	0.04±0.19	0.807
	6 months	0.02±0.29	0.18±0.29	0.138
Changes in CRT (µM) <sup>a</sup>	3 months	-27.2±125.76	48.3±96.10	0.099 <sup>c</sup>
	6 months	-16.2±157.23	133.8±171.82	0.010 <sup>c*</sup>
Mean number of injections <sup>a</sup>		4.06±1.99	4.13±1.73	0.933

<sup>a</sup>Data are presented as mean±standard deviation (SD); <sup>b</sup>Mann–Whitney U test; <sup>c</sup>Student’s t-test; \*Significant  $p$  value,  $p < 0.05$ . CRT: Central retinal thickness; BCVA: Best-corrected visual acuity; *CFH*: Complement Factor H; nAMD: Neovascular age-related macular degeneration

changes in BCVA and CRT observed for 3- and 6-month time points and in relation to the baseline measurements were determined for each *CFH* Y402H genotype (Table 2).

A significant difference in the mean BCVA was observed between the *CFH* Y402H genotypes after 6 months, with worse BCVA observed for the CC genotype ( $0.84 \pm 0.24$  logMAR) compared to the TT+CT genotypes ( $0.58 \pm 0.28$  logMAR) [ $p = 0.018$ ]. The mean CRT between the *CFH* Y402H genotypes was not significantly different after 6 months, however, the changes in CRT after 6 months compared to the baseline measurement were significantly different ( $p = 0.010$ ). The largest change in the mean CRT was observed for the CC genotype, with an increase of  $133.8 \pm 171.82$  µM after 6 months. On the contrary, no significant difference was observed in the changes in BCVA and mean number of injections between the *CFH* Y402H genotypes ( $p > 0.05$ ).

### Association between response to ranibizumab treatment and *CFH* Y402H genotypes

According to their response to ranibizumab treatment, 134 patients with nAMD were classified into responder

(n = 49) and non-responder group (n = 84), as shown in Figure 1. The mean CRT was not significantly different between the two groups ( $p > 0.05$ ), but the mean BCVA at baseline ( $p = 0.001$ ) and after 6 months ( $p = 0.005$ ) was significantly different between the two groups. At baseline, worse BCVA was observed for responder ( $0.66 \pm 0.26$  logMAR) compared to non-responder group ( $0.51 \pm 0.25$  logMAR). However, after 6 months of first injection, improved mean BCVA was observed in responder group ( $0.51 \pm 0.26$  logMAR) compared to the corresponding baseline measurement, while the mean BCVA after 6 months was worse in non-responder group ( $0.65 \pm 0.29$  logMAR) [ $p = 0.005$ ].

Next, we evaluated the mean BCVA and CRT in relation to the *CFH* Y402H genotypes in each group, as well as changes in BCVA and CRT observed for 3- and 6-month time points and in relation to the baseline measurements for each genotype in each of the two groups (Table 3). No significant difference was observed in the mean BCVA and CRT between the *CFH* Y402H genotypes in responder group ( $p > 0.05$ ). However, in non-responder group, similar to

**TABLE 3.** Visual acuity and central retinal thickness in relation to the *CFH* Y402H genotypes in responder and non-responder group

Parameter	Time point	Responders (n=49)			Non-responders (n=84)		
		CT (n=46)	CC (n=3)	<i>p</i> <sup>b</sup>	CT (n=79)	CC (n=5)	<i>p</i> <sup>b</sup>
Mean BCVA (logMAR) <sup>a</sup>	Baseline	0.65±0.26	0.69±0.27	0.949	0.50±0.25	0.64±0.27	0.242 <sup>c</sup>
	3 months	0.55±0.24	0.63±0.29	0.525	0.61±0.27	0.74±0.13	0.293
	6 months	0.48±0.24	0.70±0.36	0.240 <sup>c</sup>	0.63±0.29	0.92±0.11	0.036 <sup>c*</sup>
Mean CRT (µM) <sup>a</sup>	Baseline	407.9±155.2	315.7±65.7	0.589	362.8±123.0	304.2±69.3	0.296
	3 months	347.3±119.3	350.7±95.7	0.784	354.8±128.7	360.4±101.8	0.925
	6 months	351.1±110.3	377.0±154.5	0.569	369.7±147.5	481.4±157.5	0.097 <sup>c</sup>
Changes in BCVA (logMAR) <sup>a</sup>	3 months	-0.10±0.21	-0.06±0.22	0.35	0.11±0.19	0.10±0.15	0.840 <sup>c</sup>
	6 months	-0.17±0.25	0.01±0.34	0.056	0.13±0.25	0.28±0.24	0.150 <sup>c</sup>
Changes in CRT (µM) <sup>a</sup>	3 months	-60.7±117.9	35.0±30.4	0.351	-8.0±127.6	56.2±124.5	0.278
	6 months	-56.8±147.8	61.3±97.8	0.347	6.9±159.6	177.2±201.5	0.025 <sup>*</sup>
Mean number of injections <sup>a</sup>		4.19±1.99	3.00±1.00	0.314	3.99±2.00	4.80±1.79	0.381

<sup>a</sup>Data are presented as mean±standard deviation (SD); <sup>b</sup>Student's t-test; <sup>c</sup>Mann-Whitney U test; \*Significant *p* value, *p*<0.05. CRT: Central retinal thickness; BCVA: Best-corrected visual acuity; CFH: Complement Factor H

**TABLE 4.** Regression analysis of the *CFH* Y402H genotypes and response to ranibizumab treatment in patients with nAMD

T/C allele <sup>a</sup>	Genotype <sup>b</sup>		<i>p</i>	OR (95% CI) <sup>c</sup>	Model with least <i>p</i>
	Responders	Non-responders			
127 (47)/ 141 (53)	1/46/3	0/79/5	0.944	1.06 (0.22-5.02)	Recessive

<sup>a</sup>Data are reported as number (percentage) of alleles. <sup>b</sup>Wild-type/heterozygous/homozygous mutant genotype; <sup>c</sup>Adjusted for age and gender; CFH: Complement Factor H; nAMD: Neovascular age-related macular degeneration

the previous findings (Table 2), a significant difference was observed in the mean BCVA between the CT and CC genotypes after 6 months (*p* = 0.036), and in the changes of CRT after 6 months compared to the baseline measurement (*p* = 0.025). The mean BCVA was worse for the CC genotype (0.92 ± 0.11 logMAR) compared to the CT genotype (0.63 ± 0.29 logMAR). Similarly, an increase in CRT of 177.2 ± 201.5 µM was observed for the CC genotype compared to the CT genotype for which an increase of 6.9 ± 159.6 µm was observed. No significant difference was observed in the mean number of injections between the genotypes in both groups (*p* > 0.05).

The C risk allele was more frequent (53.0%) among the patients with nAMD compared to the low risk T allele [47.0%] (Table 4). However, in the logistic regression analysis adjusted for the confounding factors, no significant association (*p* > 0.05) was observed under the recessive model (the model with the least *p*-value) between the *CFH* Y402H genotypes and response to treatment with ranibizumab.

## DISCUSSION

In this study we investigated *CFH* Y402H polymorphism in patients with nAMD treated with ranibizumab. Specifically, we analyzed the visual prognosis of patients and their response to ranibizumab treatment in relation to the presence/absence of the Y402H homozygous risk genotype. Overall, we found no significant association between the *CFH* polymorphism and patient response to the treatment after 6-month follow-up (*p* = 0.944).

With the increasing use of anti-VEGF agents in treating nAMD, a growing number of studies has been investigating

the association between the patient response to treatment and specific gene variants [22,23]. Among the genes linked to the development of nAMD, *CFH* Y402H polymorphism has been studied most extensively, although with contradictory findings in different populations [24].

The Lucentis Genotype Study reported that a single nucleotide polymorphism (SNP) in the *CFH* gene was associated with less improvement in VA in patients receiving ranibizumab therapy [25], however, the mechanism underlying the effect of the *CFH* gene on the treatment response is still not completely understood. Nevertheless, considering the important role of *CFH* Y402H in the development of nAMD [14], investigating the association between *CFH* polymorphisms and response to treatments might provide additional insights about its function in nAMD.

In this prospective study, we investigated the association of *CFH* Y402H with the improvement in VA and response to treatment in nAMD, from both functional (BCVA) and structural (CRT) aspect. Our findings showed that, although not significantly different, patients with nAMD homozygous for the *CFH* Y402H risk allele (CC genotype) had worse VA at baseline compared to patients with CT or TT *CFH* Y402H genotype. Six months after first injection of ranibizumab, a significant difference in VA was observed between the *CFH* Y402H genotypes; the patients with the homozygous CC genotype had worse VA compared to those with the CT or TT genotype. Nevertheless, compared to the baseline measurements, worse VA was associated with all genotypes, with no significant improvement in BCVA over the 6-month follow-up period.

A similar study involving Turkish patients with CNV secondary to AMD showed that *CFH* Y402H CC accompanied a poor while TT genotype accompanied a good response in the patients undergoing ranibizumab therapy [11]. On the contrary, Lee et al. [26] reported no difference in VA between the *CFH* Y402H genotypes, after 6 and 9 months following ranibizumab treatment [26]. However, they reported that the patients with the homozygous CC genotype were more likely to require additional ranibizumab injections compared to the patients with the TT genotype. Considering the effect of the variant Y402H genotype on the regulatory role of *CFH* in the complement system, the authors suggested higher background levels of inflammation, as well as more rapid recurrence of neovascularization, as a possible pathophysiological mechanism in those patients. This could also explain why patients with the risk genotype respond differently to the treatment and require additional ranibizumab injections [26]. Veloso et al. [20] also reported that patients with nAMD carrying the CC genotype had poorer long-term functional response to intravitreal ranibizumab and required a higher mean number of injections [20].

One of the limitations of pharmacogenetic studies in AMD is the lack of uniform definition of good and poor response to the treatment [27]. In the present study, we observed a significant difference between the responder and non-responder group in the mean BCVA at baseline as well as at 6-month post-treatment time point. Furthermore, analyzing the *CFH* Y402H genotypes in relation to BCVA and CRT in each of the two groups, we showed a significant difference in the mean BCVA and in the changes of CRT between the baseline and 6-month post-treatment time point. In the non-responder group, the homozygous CC genotype was associated with worse mean BCVA and mean CRT after treatment compared to the heterozygous CT genotype, suggesting that the homozygous risk genotype might play a role in the treatment outcomes. This was also supported by the findings of Kloeckener-Gruissem et al. [18] and Dikmetas et al. [11], where AMD patients with *CFH* Y402H CC genotype had worse response to ranibizumab treatment compared to those with the CT and TT genotypes.

In the present study, in addition to BCVA, we investigated structural changes in the retina by measuring CRT before and after treatment. Our CRT analysis confirmed the possible effect of *CFH* Y402H CC genotype on a worse response to treatment, as patients with the CC genotype had a greater change in CRT after 6 months of the first injection compared to the TT and CT genotypes. Some studies [19,28,29] focused only on VA as the main outcome measure of treatment, while other studies reported conflicting results for CRT in evaluating patient response to treatment [19,20,30,31]. Nevertheless, with the advancement in OCT, CRT in combination with

other measures, is becoming a more important tool in evaluating the progress of patients with AMD [32].

Even though we observed significant differences in the BCVA and CRT between the *CFH* Y402H genotypes, the regression analysis of the responder and non-responder groups showed no association between the *CFH* Y402H genotypes and response to ranibizumab treatment.

The results of pharmacogenetic studies can be used to improve the design of clinical trials and drug development. However, studies investigating the association between specific genetic variants and patient response to treatment often use different designs, sample sizes, age and ethnic groups, and follow-up periods, which leads to inconsistent results. In the case of AMD, studies including larger samples of elderly patients and longer post-treatment follow-up periods reported more often that patients with the risk *CFH* Y402H C allele had a poorer response to treatment compared to the carriers of the low-risk T allele [11,18,19,27]. Other studies showed contradictory findings, i.e., the association between the risk CC genotype of *CFH* Y402H and response to treatment was not observed in different populations of AMD patients, based on VA and other clinical measures [22,23,28,33,34].

Our study has several limitations. The major limitation is the lack of data on different nAMD phenotypes and subtypes of CNV, which could have been used to further clarify the causes of variable treatment response. Moreover, we performed the analysis only on one gene associated with the development of AMD. The follow-up period of 6 months was also relatively short compared to some other studies. Finally, the small sample size of patients with nAMD in this study could have affected the results of the association analysis.

## CONCLUSION

This study is the first report on the association between *CFH* Y402H polymorphism and response to ranibizumab treatment in Malaysian patients with nAMD. Overall, we found no association between the *CFH* polymorphism and patient response to the treatment after 6-month follow-up. However, we observed a significant difference in the mean BCVA and changes in CRT between the *CFH* Y402H genotypes at 6-month post-treatment time point, suggesting that other factors may be involved in the progression of nAMD after treatment. Future studies with a larger sample size are required to confirm the association between the risk genotypes and ranibizumab treatment response in nAMD patients, before it can be used in genetic screening or standard treatment of AMD. Polymorphisms of other candidate genes related to AMD should also be comprehensively investigated, including the VEGF gene family, age-related maculopathy susceptibility 2 (*ARMS2*), and HtrA serine peptidase 1 (*HTRA1*).

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## DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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