DOI: 10.1590/0100-69912014006002

CFH Y402H polymorphism and response to intravitreal Ranibizumab in brazilian patients with neovascular age-related macular degeneration

Associação do polimorfismo Y402H *do gene* CFH *com a resposta terapêutica ao Ranibizumabe em pacientes portadores de degeneração macular relacionada à idade neovascular*

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A B S T R A C T

Objective: To investigate the association between *CFH* gene polymorphism and response to ranibizumab in Brazilian patients with neovascular age-related macular degeneration (AMD). **Methods:** 95 patients were genotyped for the *CFH rs1061170* (*Y402H*) single nucleotide polymorphism. Patients with neovascular AMD initially received intravitreal ranibizumab injections for three months and were retreated as needed. Visual acuity (VA) and central retinal thickness (CRT) were measured before treatment and at 1, 3, 6, and 12 months post-treatment. **Results:** For patients with the *TT* and *TC* genotypes, paired comparisons of VA showed a statistically significant improvement when the data obtained at all visits were compared with baseline. Patients homozygous for the risk genotype (*CC*) did not show a statistically significant improvement when VA obtained at visits 1, 3, 6 and 12 were compared with baseline. For all genotypes, paired comparisons of CRT showed a statistically significant improvement when the data obtained at visits 1, 3, 6 and 12 were compared with baseline. **Conclusion:** Patients with the *CC* genotype showed poorer long-term functional response to intravitreal ranibizumab.

Key words: Macular Degeneration. Genetics. Polymorphism, Genetic. Intravitreal Injections. Retina.

INTRODUCTION

Age-related macular degeneration (AMD) is a progressive disorder that affects the central retina, with primary involvement of the outer retinal layers[1]. It is considered the leading cause of severe visual acuity loss in industrialized countries and is responsible for a poor quality of life among the affected population[2,3]. Neovascular AMD occurs when a choroidal neovascular membrane grows under the retinal pigment epithelium (RPE) or between the RPE and the neurosensory retina, leading to subretinal hemorrhage or leakage of fluid and subsequent scar tissue formation. Although the etiology of AMD remains unknown, many studies have established age, smoking, and genetic predisposition as key factors for its manifestation; cardiovascular risk factors (such as hypertension and hyperlipidemia) were considered inconsistent contributors to disease presentation[4-6]. The AMD-associated genes might interact with other genes or nongenetic risk factors to produce the clinical phenotypes. Recent studies have shown that some genetic single nucleotide polymorphisms (SNPs) are associated with AMD[1,7,8]. A genetic variation in the *complement factor H* (*CFH*) gene on chromosome 1q32 is one of the most studied gene polymorphisms related to AMD. This polymorphism (*rs1061170*) results in a tyrosine-to-histidine substitution at the amino acid position 402 (*Y402H*) in the CFH protein and confer an increased risk for the development of AMD[9,10].

Interindividual differences in drug response are partially attributed to genetic variations, which have led several groups to conduct pharmacogenetic studies with a hope of offering personalized treatment for AMD patients. Some authors have studied the effect of genotypes on the response to nutritional supplements, showing a positive association[11]. Other have evaluated the relationship between *CFH* genotypes and photodynamic therapy (PDT), with controversial results[12-16].

The association between gene polymorphisms and response to anti-vascular-endothelial growth-factor (VEGF) therapy for neovascular AMD has also been studied[12-27]. Intravitreal injections of anti-VEGF agents were the first treatment that consistently improved visual acuity (VA) in a large number of patients, representing a remarkable advance in the treatment of neovascular AMD. However, there is a broad range of response rates to anti-VEGF therapy, and genetic variants may be partially responsible[6]. Several

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reports evaluated *CFH* genotype association with the response to the anti-VEGF agents bevacizumab and ranibizumab, some of them suggesting a potential pharmacogenetic relationship[17-26].

The purpose of this study was to investigate the association between *CFH* Y402H (*rs1061170*) SNP with response to ranibizumab therapy in neovascular AMD for the first time in a Brazilian population.

METHODS

This study was part of a retrospective review of prospectively acquired data of patients with AMD that included the identification of *CFH rs1061170* SNP and its relationship with the development of the disease, and the therapeutic response to anti-VEGF treatment. All subjects were informed about the nature of the study and signed a written consent in accordance with the guidelines of The Declaration of Helsinki. The Ethics in Research Committees of both the Federal University of Minas Gerais and the Institute of Vision in Belo Horizonte, Brazil, approved the study.

All patients with AMD diagnosed between 2008 and 2012 at the Institute of Vision underwent a complete ophthalmological examination, including biomicroscopy, retinography, fluorescein angiography (FA) and optical coherence tomography (OCT). When indicated, indocyanine green angiography was performed for better evaluation of the neovascular AMD subtypes. All patients had a blood sample taken to study the genetic SNP possibly associated with this disease. Inclusion criteria were: (a) age > 50 years; (b) diagnosis of neovascular AMD; (c) visual acuity better than 20/400 (If both eyes were affected, the one with the worse VA was selected); (d) loading dose with three intravitreal injections of ranibizumab (Lucentis; Novartis, Basel, Switzerland, and Genentech, Inc., South San Francisco, CA), administered one time per month over three months; and (e) follow-up period of at least 12 months. Exclusion criteria were: (a) choroidal neovascularization secondary to any cause other than AMD; (b) previous treatment for neovascular AMD; (c) patients with indication for combined treatment; (d) polypoidal choroidal vasculopathy; (e) eyes previously submitted to posterior vitrectomy; and (f) other diseases that could affect VA.

We performed all intravitreal anti-VEGF injections of ranibizumab (0.5 mg/0.05 mL) in the operating room, with aseptic technique, including the prophylactic use of topical 5% iodopovidone. All patients were subjected to a treatment protocol that included a loading dose with three intravitreal injections of ranibizumab at one-month intervals. After the third dose, they followed a *pro re nata* regimen. Retreatment criteria were: (a) persistence or increase of intra- or subretinal fluid; (b) increase of RPE detachment; (c) worsening of at least one line of VA; and (d) new subretinal hemorrhage. All patients were retreated with ranibizumab.

Best-corrected visual acuity (BCVA) and central retinal thickness (CRT), obtained using spectral domain optical coherence tomography (Spectralis OCT™ [*Heidelberg Engineering*, Heidelberg, Germany]), were measured at baseline and at one month after each intravitreal injection. We used the automated segmentation of retinal boundaries and any segmentation error was corrected manually. We determined CRT based on central 1-mm subfield thickness. Snellen VA was recorded in a standardized manner for all patients during all visits and, for statistical analysis purposes, was converted to the logarithm of minimal angle of resolution (logMAR) values. VA, OCT, and intravitreal injections were performed by different investigators, in a double-masked fashion.

Genotyping

Genomic DNA was isolated from whole blood based on the high salt method of Lahiri and Nurnberger[25]. We selected one SNP, which was genotyped using TaqMan® SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA). The probe used corresponds to the *rs1061170* (*Y402H*) *CFH* SNP. The polymorphism was chosen using the Hapmap database (www.hapmap.org). We performed retyping of 10% of the whole sample for quality control[28].

Genotypings were read using PCR-Realtime in the allelic discrimination mode (Strategene Mx3005 – MxPro QPCR- Software, 2007). PCR protocols followed the instructions for use of the TaqMan® Genotyping Master Mix (Applied Biosystems), as follows: 3.5 µl of mix, 0.1 µl of probe, 3.4 µl of deionized water, and a 1.0 µl DNA concentration 50 ng/µl to a total volume of 8 µl. PCR conditions were: 1 cycle (10 min at 95°C) and 50 cycles (95°C at 15 s, 60 °C 1 min).

Statistical analysis

We calculated descriptive statistics for all demographic and clinical variables. When quantitative variables were compared with qualitative variables with three categories, we employed the F (ANOVA) test if the values were sampled from a Gaussian distribution (verified by Hosmer-Lemeshow test), and otherwise, by a Kruskal-Wallis test. We performed comparisons between proportions of qualitative variables by the chi-square test. Paired comparisons were made using the paired *t* test if the values were sampled from a Gaussian distribution (verified by Hosmer-Lemeshow test), and otherwise, by a Wilcoxon test.

RESULTS

A total of 601 eyes of 370 AMD patients were evaluated and 95 met the inclusion criteria. The mean age of the 95 patients was 73.9 ± 8.5 years (range 54-91) and

50 (52.6%) were female. The mean pre-treatment BCVA was 0.58 ± 0.3 logMAR and the mean pre-treatment CRT was 342 ± 90 ìm. The pre-treatment BCVA, CMT and gender distribution were not statistically different for the three different genotypes (Table 1). Patients with *TT* genotype, however, were older than patients with *TC* and *CC* genotypes (p = 0.0248). No serious local and nor systemic adverse effect was noted in any case. Twenty-four patients (25.3%) were homozygous for the risk allele (*CC*), 52 (54.7%) had at least one risk allele (*TC*), and 19 (20.0%) were homozygous for the T allele (*TT*).

For all 95 patients, paired comparisons of VA showed statistically significant improvement when the data obtained at visits 1, 3, 6, and 12 were compared with baseline (Table 2). For patients with the *TT* and *TC* genotypes, paired comparisons of VA also showed statistically significant improvement when the data obtained at all visits were compared with baseline. However, patients with the *CC* genotype did not show statistically significant improvement when VA obtained at visits 1, 3, 6 and 12 were compared with baseline.

For the whole group of patients and for each genotype individually analyzed, paired comparisons of CRT showed statistically significant improvement when the data obtained at visits 1, 3, 6, and 12 were compared with baseline (Table 3).

The mean number of intravitreal injections required between the third and twelfth month was 1.6 ± 0.8 (median: 2.0) for patients homozygous for the *T* allele; 2.5 ± 1.7 (median: 2.0) for patients with the *TC* genotype, and 3.0 ± 2.3 (median: 3.0) for patients with the *TT* genotype (p = 0.05; Kruskal-Wallis test).

DISCUSSION

Genetic factors are known to play a major role in the pathogenesis of AMD and have been suggested to influence the response to different modalities of AMD therapy, including oral antioxidants, PDT, and anti-VEGF agents[11-27]. The majority of the studies evaluated *CFH rs1061170* (*Y402H*) SNP. Klein *et al* made a retrospective analysis of participants of the Age-Related Eye Disease Study (AREDS) to investigate the possible association between the response to oral antioxidants and zinc with genetic polymorphisms. There was s greater reduction in AMD progression (68%) in individuals with the low-risk *CFH TT* genotype compared with those with the high-risk *CC* genotype (11%), suggesting limited benefits of supplementary diets in individuals with this genetic background[9].

Other authors have studied the effect of the CFH *rs1061170* (*Y402H*) on the response to PDT, with controversial results[10-14]. Goverdhan *et al* showed that patients homozygous for the *CFH* high-risk allele seem to have worse outcomes after PDT[10]. Brantley *et al.* also found a potential relationship between *CFH* genotype and response to PDT. However, they showed that patients homozygous for the *CFH* non-risk allele (*TT*) fared significantly worse with PDT than those with the *CFH TC* and *CC* genotypes[11]. Other studies did not show significant association between *CFH* polymorphism and PDT response for neovascular AMD[12-14].

Recent works have demonstrated the association between *CFH rs1061170* (*Y402H*) SNP and the response to intravitreal injections of the anti-VEGF agents bevacizumab and ranibizumab[15-24]. Brantley *et al.* evaluated patients that underwent intravitreal injections of bevacizumab at six-week intervals until there was no longer evidence of active neovascularization. The authors showed that, after 6 months, VA was significantly worse in the *CFH CC* genotype than for the *CFH TC* or *TT* genotypes[15]. Lee *et al.* investigated whether *CFH* genotypes had an effect on the treatment of neovascular AMD with ranibizumab. Intravitreal injection was performed at baseline and subsequent injections were performed as needed. No difference was found in VA outcomes after ranibizumab treatment among the different *CFH* genotypes. Nevertheless, over nine months, patients with both risk alleles received approximately 1 more intravitreal injection[16]. Nischler *et al.* prospectively evaluated AMD patients treated with intravitreal bevacizumab at six-week intervals until there was no longer evidence of active

Table 1 - Pre-treatment findings according to *CFH* genotypes.

	Genotypes			p-value
	TT (n=19)	*TC* (n=52)	*CC* (n = 24)	
Age*	78.4 ± 4.5 (78)	73.1 ± 9.8 (75)	72.0 ± 6.4 (72)	0.0248***
Male/Female	6 / 13	24 / 28	9 / 15	0.4993**
VA* - logMAR	0.63 ± 0.3 (0.8)	0.57 ± 0.3 (0.5)	0.53 ± 0.4 (0.4)	0.3788***
CMT* - µm	351 ± 89 (332)	326 ±74 (305.5)	372 ±116 (337)	0.3372***

VA: visual acuity; CMT: central macular thickness; logMAR: logarithm of minimal angle of resolution.
***: mean ± standard deviation (median).*
***: Chi-square test.*
****: Kruskal-Wallis test.*

neovascularization. Patients homozygous for the risk allele showed worse functional response to treatment. For CRT, all genotypes showed statistically significant improvement[17]. Imai *et al* found an association of *CFH rs1061170* (Y402H) SNP with VA improvement after one intravitreal bevacizumab injection. Heterozygotes showed worse response to treatment one and three months after treatment[18]. Smailhodzic *et al.* studied *CFH rs1061170* (Y402H) SNP and found a significant decrease in VA after ranibizumab treatment in the group carrying all six high-risk alleles in *CFH*, *LOC387715,* and *VEGF*, when compared with the remaining AMD patients. Carriers of all six risk alleles demonstrated a mean loss of 10 Early Treatment Diabetic Retinopathy Study (ETDRS) letters after treatment, whereas all other allele groups demonstrated an improvement in VA after treatment. They concluded that there was a cumulative effect of high-risk alleles, with poor response rates to intravitreal ranibizumab treatment in combination with a younger age of neovascular AMD onset[19]. Kloeckener-Gruissem *et al.* studied the influence of *CFH Y402H* SNP in patients submitted to intravitreal ranibizumab. Further injections were performed only if signs of activity were still present. After 12 months of follow-up, patients homozygous for the risk-allele (*CC*) showed worse response to treatment than patients with the *CT* and *TT* genotypes[20]. Mckibbin *et al* evaluated caucasian patients with neovascular AMD treated with ranibizumab and followed for 6 months. They

Table 2 - Comparison of mean visual acuity according to *CFH* genotypes, between baseline and at 1, 3, 6 and 12 months after the beggining of the treatment.

	Baseline	1 month	3 months	6 months	12 months
TT genotype *(n = 19)*					
VA* - logMAR	0.63 ± 0.3 (0.8)	0.57 ± 0.3 (0.6)	0.54 ± 0.3 (0.5)	0.46 ± 0.3 (0.4)	0.44 ± 0.3 (0.3)
P-value		0.03**	0.02**	0.02**	0.01**
TC genotype *(n = 52)*					
VA* - logMAR	0.57 ± 0.3 (0.5)	0.49 ± 0.3 (0.4)	0.46 ± 0.4 (0.4)	0.50 ± 0.4 (0.5)	0.57 ± 0.5 (0.5)
P-value		0.004**	0.0007**	0.009**	0.03**
CC genotype *(n = 24)*					
VA* - logMAR	0.53 ± 0.4 (0.4)	0.61 ± 0.7 (0.3)	0.57 ±0.6 (0.25)	0.61 ± 0.7 (0.3)	0.52 ± 0.6 (0.3)
P-value		0.76**	0.38**	0.85**	0.45**
CC. CT and TT genotypes					
VA* - logMAR	0.58 ± 0.3 (0.5)	0.54 ± 0.4 (0.4)	0.51 ± 0.4 (0.4)	0.52 ± 0.4 (0.4)	0.53 ± 0.5 (0.4)
P-value		0.0019	<0.0001	0.0023	0.0017

VA: visual acuity; logMAR: logarithm of minimal angle of resolution.
**: mean ± standard deviation (median).*
******: Wilcoxon *test.*

Table 3 - Comparison of mean central retinal thickness according to *CFH* genotypes, between baseline and at 1, 3, 6 and 12 months after the beggining of the treatment.

	Baseline	1 month	3 months	6 months	12 months
TT genotype (n = 19)					
CRT* - µm	351 ± 89 (332)	298 ± 68 (315)	268 ± 64 (239)	272 ± 48 (272)	261 ± 52 (262)
P-value		0.0013**	0.0004**	0.0017**	0.0002**
TC genotype (n = 52)					
CRT* - µm	326 ±74 (305.5)	286 ±65 (286.5)	280 ± 63 (281)	287 ± 72 (275)	276 ±69 (250.5)
P-value		<0.0001**	<0.0001**	0.0004**	<0.0001**
CC genotype (n = 24)					
CRT* - µm	372 ± 116 (337)	344 ± 100 (331)	324 ±101 (299.5)	286 ±110 (265)	298 ±129 (263.5)
P-value		0.0126**	0.0053**	<0.0001**	0.0001**
CC. CT and TT genotypes					
CRT* - µm	342 ± 90 (325)	303 ± 78 (296)	289 ± 77 (281)	284 ± 80 (281)	279 ± 86 (258)
P-value		<0.0001**	<0.0001**	<0.0001**	<0.0001**

CRT: central retinal thickness;
**: mean ± standard deviation (median).*
******: Wilcoxon *test.*

found a trend towards a more favourable outcome with the higher AMD risk genotypes in *CFH Y402H* SNP[21]. Menghini *et al*. recently studied eyes treated with ranibizumab for neovascular AMD and showed that the *CT* genotype at *CFH rs1061170* (Y402H) was a significant predictor for a favorable VA outcome at 12 and 24 months[22]. Yamashiro et al investigated the role of *CFH rs1061170* (*Y402H*) SNP in AMD patients treated with intravitreal ranibizumab injections and followed for more than 1 year after treatment. There was no clear association between the studied SNP and responsiveness to ranibizumab treatment[23]. The largest study conducted so far involved participants of the Comparison of AMD Treatments Trials (CATT) recruited through 43 clinical centers. Each patient was genotyped for SNPs *rs1061170* (*CFH*), *rs10490924* (*ARMS2*), *rs11200638* (*HTRA1*) and *rs2230199* (*C3*). There were no identified statistically significant differences in response by genotype for any of the clinical measures studied. Specifically, there were no high-risk alleles that predicted final VA or change in VA, the degree of anatomic response or the number of injections. Furthermore, a stepwise analysis failed to show a significant epistatic interaction among the variants analyzed; that is, response did not vary by the number of risk alleles present. The lack of association was similar whether patients were treated with ranibizumab or bevacizumab or whether they received monthly or *pro re nata* dosing[24].

In our study, gender, VA, and CRT analyzed at baseline showed no differences between the three *CFH* genotypes, which means that the sample was quite well balanced. However, patients homozygous for the *T* allele were older (p = 0.0248), suggesting that patients that present the risk allele may develop the disease earlier in life. Since our study was not designed with this purpose, other ones need to investigate factors associated with early onset of the disease. Patients with polypoidal choroidal vasculopathy were excluded from this study, since this clinical entity has some characteristics that differ from the typical AMD subtypes, including a worse response to anti-VEGF drugs[29,30]. All patients underwent a treatment protocol that involved three intravitreal injections at one-month intervals and then as needed, as previously suggested[31].The present study showed that patients with the *CC* genotype had a worse functional response to ranibizumab therapy during one year of follow-up.

We consider that the analysis of treatment response just after one and three months is especially important, since it encompasses patients that received the same number of injections, regardless of genetic variants. After the loading dose, it becomes more difficult to analyze the results when a *pro re nata* regimen is employed. In this situation, both treatment response and the number of retreatments need to be analyzed, since eyes that do not respond well to therapy usually require more injections. Our study showed that the mean number of intravitreal injections required was higher for patients homozygous for the *C* allele. Since the treatment was given with a loading dose of three consecutive injections followed by a maintenance phase with repeated injection only as required, the results may not be applicable to patients treated with regular monthly injections. It is possible that, for patients treated with fixed monthly injections, even more resistant cases might have a final visual outcome similar to those that initially present a good response. Thus, we can speculate that the identification of a possible association between genetic polymorphisms and response to therapy could be useful, as it might identify subjects who are more refractory to treatment and therefore may receive monthly injections rather than a *pro re nata* regimen. Conversely, it could identify individuals whose therapeutic response was so favorable that it would allow them to be treated with longer intervals between injections.

Most pharmacogenetic studies involving neovascular AMD therapy included a limited number of patients and used different treatment regimens. This could explain, at least in part, the different results. Differences in the studied population could also be partially responsible. It should be noted that this is the first pharmacogenetic study conducted in Brazil, a multiethnic country. Even though our study also presents some limitations, including sample size, it showed a significant correlation between *CFH* gene polymorphism and the treatment outcome in neovascular AMD, with the risk allele carrying a worse functional prognosis, in agreement with other similar published articles. Future evaluation of the interaction between environmental factors, *CFH* gene polymorphism and other genetic variants will be important to understand the different therapeutic responses in AMD patients.

It is possible that some genetic polymorphisms could influence anti-VEGF therapy and lead to a customized treatment for AMD in the near future. Knowledge of prognostic factors before initiating treatment has the potential to reduce side effects and improve the quality of life of our patients, since it adds a new criterion for a better treatment indication and a personalized regimen. A greater understanding of pharmacogenetics will allow the approach to this disease to be chosen or modified based on the genetic profile of the patients.

R E S U M O

Objetivo: *investigar a associação entre polimorfismo do gene* CFH *e a resposta terapêutica ao ranibizumabe na degeneração macular relacionada à idade (DMRI) neovascular.* ***Métodos:*** *noventa e cinco pacientes foram submetidos à genotipagem para identificação do polimorfismo* rs1061170 *(Y402H) do gene* CFH. *Pacientes portadores de DMRI neovascular receberam inicialmente três injeções intravítreas de ranibizumabe com intervalo mensal entre elas. A partir de então, foram retratados de acordo com a necessidade. Acuidade visual (AV) e espessura macular central (EMC) foram medidas antes e 1, 3, 6 e 12 meses após o início do tratamento.* ***Resultados:*** *para pacientes portadores dos genótipos* TT *e* TC*, a análise pareada da AV mostrou melhora estatisticamente significativa quando os dados obtidos em todas as visitas foram comparados com aqueles obtidos antes do início do tratamento. Para pacientes homozigotos para o alelo de risco (CC), não houve diferença estatisticamente significativa quando a AV obtida nas visitas 1, 3, 6 e 12 foi comparada com aquela obtida antes do início do tratamento. Para todos os genótipos, a análise pareada da EMC mostrou melhora estatisticamente significativa em todas as avaliações.* ***Conclusão:*** *pacientes portadores do genótipo* CC *apresentaram pior resposta funcional em longo prazo após o tratamento com ranibizumabe intravítreo.*

Descritores: *Degeneração Macular. Genética. Polimorfismo Genético. Injeções Intravítreas. Retina.*

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Received 29/11/2013
Accepted for publication 07/02/2014
Conflict of interest: none.
Source of funding: none.

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