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Pharmacogenetics of Complement Factor H (Y402H) and treatment of exudative age-related macular degeneration with ranibizumab

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Abstract

Aims—To determine whether complement factor H (*CFH*) genotypes have a pharmacogenetic effect on the treatment of exudative age-related macular degeneration (AMD) with ranibizumab.

Methods—A retrospective study of 156 patients with exudative AMD treated with intravitreal ranibizumab monotherapy was conducted. AMD phenotypes were characterized by clinical examination, visual acuity, fundus photography, fluorescein angiography, and injection timing. Patients received intravitreal ranibizumab injections as part of routine ophthalmologic care and were followed for a minimum of nine months. Each patient was genotyped for the single nucleotide polymorphism rs1061170 (Y402H) in the *CFH* gene.

Results—Baseline lesion size and angiographic type, as well as mean visual acuities at baseline, 6 months, and 9 months were similar among the three *CFH* genotypes. Over 9 months, patients with both risk alleles received approximately one more injection ($p = 0.09$). In a recurrent event analysis, patients homozygous for the *CFH* Y402H risk allele had a 37% significantly higher risk of requiring additional ranibizumab injections ($p = 0.04$)

Conclusions—In our cohort, response to treatment of AMD with ranibizumab differed according to *CFH* genotype, suggesting that determining patients' *CFH* genotype may be helpful in the future in tailoring treatment for exudative AMD with intravitreal ranibizumab.

Keywords

Complement Factor H; Ranibizumab; Age-Related Macular Degeneration; Pharmacogenetics

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INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of irreversible vision loss in individuals over the age of 50 years in the Western world.[1] The vast majority of AMD-related vision loss results from exudative (neovascular) AMD, characterized by invasion of blood vessels into subretinal spaces. Along with environmental risk factors such as smoking and hypertension, genetics is a primary contributor to AMD susceptibility.[2, 3] Recently, a thymine-to-cytosine (T-to-C) transition in the complement factor H gene (*CFH*, Y402H) was found to be strongly associated with AMD.[4–6] This polymorphism has also been associated with exudative AMD[7] and AMD progression.[8]

A small number of studies have investigated the potential pharmacogenetic relationship between the *CFHY402H* variant and therapeutic response in AMD. Analyzing data from the Age Related Eye Disease Study (AREDS), Klein *et al.* recently demonstrated that *CFH* Y402H genotypes may be predictive of treatment response to zinc supplementation.[9] Further studies have shown an effect of *CFHY402H* genotype on response of exudative AMD to photodynamic therapy.[10–12] Recently, we reported that patients with the CC genotype (two *CFHY402H* variant alleles) demonstrated less visual acuity improvement with intravitreal injections of the anti-vascular endothelial growth factor (anti-VEGF) agent bevacizumab than patients with the TC and TT genotypes.[13] Since these studies, a second anti-VEGF therapy, ranibizumab (Lucentis, Genentech, Inc. (US)), has been approved for the treatment of exudative AMD and is now considered standard of care.

The purpose of this study is to determine if there is an association between *CFH* genotypes and therapeutic outcomes with ranibizumab, controlled for variable patient follow-up, smoking pack-years and the presence of diabetes and hypertension.

METHODS

Patients, clinical examination, and treatment

This retrospective cohort study was approved by the Washington University Human Research Protection Office and the Barnes Retina Institute Study Center. Research adhered to the tenets of the Declaration of Helsinki and was conducted in accordance with Health Insurance Portability and Accountability Act regulations. All participants were enrolled from the clinical offices of the Barnes Retina Institute and signed written informed consent prior to participation. Patients were identified through chart review, and only Caucasian patients with no previous anti-VEGF treatment in either eye and no previous AMD therapy of any kind in the study eye were included.

Mouthwash samples for genotyping were collected from 178 patients with a diagnosis of exudative AMD who were undergoing treatment for an active neovascular lesion with intravitreal ranibizumab. For each patient, an intravitreal injection of 0.5 mg ranibizumab was performed at the initial presentation of an active choroidal neovascular complex, and subsequent injections were performed at the discretion of the physician based on clinical examination and ancillary test findings, including subretinal fluid on optical coherence tomography or leakage on fluorescein angiography. For each patient, an intravitreal injection of 0.5 mg ranibizumab was performed at the initial presentation of an active choroidal neovascular complex, and subsequent injections were performed at the discretion of the treating physician based on clinical examination and ancillary test findings, including new macular hemorrhage, intraretinal or subretinal fluid on optical coherence tomography, or leakage on fluorescein angiography. Each patient was followed for a minimum of nine months for inclusion in the study.

During the collection of the mouthwash sample, information regarding smoking history, diabetes, and hypertension was obtained. Modeled after Schmidt *et al.*, [14] regular smoking history was assessed by two questions: (1) "Have you smoked 100 cigarettes in your lifetime?" and (2) "Did you smoke cigarettes at least once a week?" Patients who answered "yes" to both questions were then asked the average number of cigarettes a day, the year that they started smoking, and the year that they stopped. This information was then converted to smoking pack years by the formula:

$$\text{smoking pack-years} = (\text{cigarettes per day})(\text{years smoked})/(20 \text{ cigarettes per pack})$$

Diabetes history was assessed by asking if the patient had a diagnosis or history of diabetes mellitus. Hypertension history was assessed by asking the patient whether they had a history of high blood pressure. Patients who responded "no" were confirmed by asking if they were currently taking any medications to lower blood pressure.

AMD phenotypes were characterized by clinical examination including dilated fundus exam, fundus photography, and fluorescein angiography. Fluorescein angiograms (FAs) were obtained digitally with a Zeiss fundus camera and imaging software (OIS, Sacramento, CA) upon initial presentation of active choroidal neovascularization. Neovascular lesions were classified as predominantly classic, minimally classic, or occult by a retina specialist masked to genotype. Lesion size, including disc area and greatest linear dimension (GLD), was calculated. Snellen visual acuity was recorded in a standardized manner for all patients at initial presentation and at follow-up visits. For all calculations and comparisons, Snellen acuities were converted to log Minimal Angle of Resolution (logMAR) values.

DNA preparation and genotyping

Participants provided buccal tissue samples by expectorating into 50 ml conical tubes (Falcon; BD Biosciences, San Jose, CA) after vigorously rinsing for 30 seconds with ~20 ml Scope mouthwash (Procter & Gamble, Cincinnati, OH). Genomic DNA was prepared from buccal cells using the Gentra Puregene Buccal Cell kit (qiagen.com, Valencia, CA) and quantified by absorbance at 260 nm (GeneQuant pro, GE Healthcare, Buckinghamshire, UK). Genotyping for a single nucleotide polymorphism in the *CFH* gene (Y402H, rs1061170 T/C) was performed by Sequenom MassARRAY technology (sequenom.com, San Diego CA).

Data analysis

Descriptive statistics for all demographic and clinical variables were calculated and comparisons made using the ANOVA test for means with continuous data (e.g., age, visual acuity) and the chi-square test for categorical data (e.g. gender). Values for lesion area and GLD on FA were log-transformed to ensure normality of distribution. Four lesions with sizes that were outliers of more than 2 standard deviations from the mean were excluded. The association between genotype and post-treatment visual acuities were assessed using two generalized linear modeling techniques at 6 and 9 months separately, with visual acuities expressed in logMAR units. Adjustments were made for baseline VA, and any predictors that trended towards significance ($p < 0.10$) by univariate analysis were included in the multivariate models. Genotype was included in both models with a dummy variable for the homozygous candidate allele (i.e., TT for CFH) and was determined to be a significant predictor if the p value associated with the coefficient was less than 0.05. Univariate analysis was performed for the total number of injections. To further examine the temporal relationship of injections by genotype, a conditional approach by Cox regression analysis for modeling recurrent events was built with time between injections after the first 70 days as the outcome and genotypes as the main effect. The first 70 days were excluded from the recurrent event analysis since most patients received a three injection induction. In

addition, to find any differences in follow-up by genotype, the same recurrent event analysis was performed for the intervals between follow-up appointments. Statistics were performed with SAS (version 9.1; SAS Institute, Cary NC USA) and SPSS (version 15; SPSS Inc., Chicago IL USA). For all statistical analyses, $p < 0.05$ was considered to be statistically significant.

RESULTS

Of the 178 mouthwash samples collected from the patients of nine retinal specialists, 156 (88%) produced sufficiently high quality DNA for genotypic analysis. For the *CFHY402H* polymorphism, 37 patients (24%) were TT, 71 (45%) were TC, and 48 (31%) were CC. The prevalence of the C risk allele was 54%. Baseline characteristics are described in Table 1, including lesion characteristics on the 118 available FAs. No significant differences were found among the genotype distributions for any of the predictor variables.

To examine the effect of genotype on treatment outcomes with ranibizumab, we considered the post-treatment visual acuities at 6 and 9 months after adjusting for pretreatment visual acuity. The *CFH* genotypes were not found to significantly affect the post-treatment VA at 6 months ($p = 0.38$) or 9 months ($p = 0.70$) (Table 2). However when the mean number of injections over the 9 month period was examined, we did observe a trend in the number of injections required over a 9 month period among the three genotypes, with the TT group receiving the fewest injections ($p = 0.09$).

Given the trend toward requiring additional ranibizumab injections with the presence of the *CFHY402H* risk allele, we performed a recurrent event analysis to determine if this polymorphism affected the interval between required injections. We found that, over a nine month period, patients with the heterozygote variant (TC) tended to have a 25% higher risk of needing another injection than patients with the TT genotype. Furthermore patients with the homozygote variant (CC) were found to have a 37% significantly higher risk ($p = 0.04$) of needing another injection (Table 3). When examining the intervals between follow-up appointments in the same manner, we found that there was no difference between how often patients with the TC genotype were seen in follow-up compared to patients with the TT genotype (HR: 1.00, $p = 0.95$) nor between how often patients with the CC genotype were seen in follow-up compared to patients with the TT genotype (HR: 1.03, $p = 0.76$).

CONCLUSIONS

The burgeoning field of pharmacogenetics brings clinicians the ability to tailor pharmacotherapy to patients' individual genetic variations. In order to suggest a change in clinical practice recommendations, studies must first prove that therapeutic differences correlate with genotypes.[15] In this retrospective study of patients with exudative AMD treated with ranibizumab monotherapy, we found that patients homozygous for the variant genotype for *CFH* (CC) were more likely to require reinjection than the TT genotype. We also found that over a period of nine months, there was a trend toward requiring more total injections with the variant genotypes than with the TT genotype. In addition, we found that there was no difference in follow-up appointments by genotype, indicating that patients in each cohort were followed without bias and that the difference in injection timing was not due to differences in follow-up. Finally, we found a stepwise increase in risk of additional injections, as patients with the TC genotype had a 25% increased risk ($p = 0.12$), and patients with the CC genotype had a 37% statistically significant higher risk ($p = 0.04$), suggesting a possible genotype-dose dependent pharmacogenetic effect.

We found no difference in visual acuity outcomes after ranibizumab treatment at 6 and 9 months among the different genotypes, in contrast to our previous study with bevacizumab. [13] Several factors may account for this difference. First, the current study had more stringent inclusion criteria, a longer follow up period, and controlled for variability in the length of follow-up. Second, the patient cohort in the current study had substantially better baseline VA (0.85 logMAR) compared to the baseline VA of 1.02 logMAR in the bevacizumab study, potentially affecting the post-treatment outcome. This difference in VA at baseline most likely reflects the current wide usage of ranibizumab for all exudative AMD patients, compared to more selective off-label use of bevacizumab at the time of the previous study. Finally, while the two drugs are similar in their mode of action and structure, treatment response could possibly be affected differently by *CFH* genotypes.

One plausible pathophysiological explanation for our primary findings is that patients with the variant Y402H genotype in *CFH* have higher background levels of inflammation since *CFH* is the primary regulator of the alternative complement system. While it has been hypothesized that this variation may be a molecular mechanism for the onset of neovascular AMD, variations in the *CFH* gene may continue to affect the disease progression and lead to more rapid recurrence of neovascularization. Thus patients with the variant genotypes may respond differently to treatment, and require additional injections of ranibizumab.

While our study did find a potential pharmacogenetic association between *CFH* Y402H genotypes and efficacy of ranibizumab therapy, there are limitations to the study method. Since the study was retrospective in design, the predictive value of *CFH* genotypes could not be tested. The follow-up of patients was not consistent, and thus the cohorts at 6 and 9 months were not identical. While this could influence the results of the visual acuity outcomes, the recurrent event analysis is a type of survival analysis and thus is robust to variable follow-up and censored data. Finally, since there was not a placebo control group, the observed effects could potentially be based on the pathophysiology of natural disease rather than true pharmacogenetic effect.

In conclusion, we describe here the first association of a pharmacogenetic effect of the *CFH* Y402H variations with ranibizumab in exudative AMD. We found that response to treatment of AMD with ranibizumab differed according to *CFH* genotype, suggesting that further investigations are warranted to see if patients with the CC and TC genotype may need to be monitored more closely for disease recurrence than the TT genotype. Prospective studies are needed to confirm this association before any recommendation for genetic screening or change current standard of care with ranibizumab can be made.

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REFERENCES

1. Friedman DS, O'Colmain BJ, Muñoz B, et al. Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol.* 2004; 122:564–572. [PubMed: 15078675]

2. Klein R, Klein BE, Linton KL, et al. The Beaver Dam Eye Study: the relation of age-related maculopathy to smoking. *Am J Epidemiol.* 1993; 137:190–200. [PubMed: 8452123]
3. Scholl HP, Fleckenstein M, Charbel Issa P, et al. An update on the genetics of age-related macular degeneration. *Mol Vis.* 2007; 13:196–205. [PubMed: 17327825]
4. Klein RJ, Zeiss C, Chew EY, et al. Complement factor H polymorphism in age-related macular degeneration. *Science.* 2005; 308:385–389. [PubMed: 15761122]
5. Edwards AO, Ritter R, Abel KJ, et al. Complement factor H polymorphism and age-related macular degeneration. *Science.* 2005; 308:421–424. [PubMed: 15761121]
6. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. *Science.* 2005; 308:419–421. [PubMed: 15761120]
7. Souied EH, Leveziel N, Richard F, et al. Y402H complement factor H polymorphism associated with exudative age-related macular degeneration in the French population. *Mol Vis.* 2005; 11:1135–1140. [PubMed: 16379025]
8. Seddon JM, Francis PJ, George S, et al. Association of CFH Y402H and LOC387715 A69S with progression of age-related macular degeneration. *JAMA.* 2007; 297:1793–1800. [PubMed: 17456821]
9. Klein ML, Francis PJ, Rosner B, et al. CFH and LOC387715/ARMS2 genotypes and treatment with antioxidants and zinc for age-related macular degeneration. *Ophthalmology.* 2008; 115:1019–1025. [PubMed: 18423869]
10. Goverdhan SV, Hannan S, Newsom RB, et al. An analysis of the CFH Y402H genotype in AMD patients and controls from the UK, response to PDT treatment. *Eye.* 2008; 22:849–854. [PubMed: 17464302]
11. Seitsonen SP, Jarvela IE, Meri S, et al. The effect of complement factor H Y402H polymorphism on the outcome of photodynamic therapy in age-related macular degeneration. *Eur J Ophthalmol.* 2007; 17:943–949. [PubMed: 18050121]
12. Brantley MA, Edelstein SL, King JM, et al. Association of complement factor H and LOC387715 genotypes with response of exudative age-related macular degeneration to photodynamic therapy. *Eye.* 2008 epub.
13. Brantley MA, Fang AM, King JM, et al. Association of complement factor H and LOC387715 genotypes with response of exudative age-related macular degeneration to intravitreal bevacizumab. *Ophthalmology.* 2007; 114:2168–2173. [PubMed: 18054635]
14. Schmidt S, Hauser MA, Scott WK, et al. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. *Am J Hum Genet.* 2006; 78:852–864. [PubMed: 16642439]
15. Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science.* 1999; 286:487–491. [PubMed: 10521338]

TABLE 1

Baseline and lesion characteristics by *CFHY402H* genotypes.

	TT (n = 37)	TC (n = 71)	CC (n = 48)	All (n = 156)	Significance <i>p</i> =
Gender (males (%))	13 (35%)	23 (32%)	14 (29%)	50 (32%)	0.84
Eye (OD (%))	19 (51%)	37 (52%)	23 (48%)	79 (51%)	0.90
Age (mean)	84	83	83	83	0.57
Diabetes (n (%))	6 (16%)	17 (24%)	8 (17%)	31 (20%)	0.51
Hypertension (n (%))	24 (65%)	42 (59%)	31 (65%)	97 (62%)	0.78
Smoking pack years (mean)	13.7	21.2	27.7	21.4	0.21
Available FAs (n)	29	52	37	118	
Occult type (n (%))	20 (69%)	26 (50%)	19 (51%)	65 (55%)	0.28
Lesion size (mean disk area)	2.6	2.9	1.8	2.5	0.23
Greatest linear dimension (mean mm)	2618	2744	2206	2545	0.22

TABLE 2Visual acuity outcomes and number of ranibizumab injections by *CFHY402H* genotype

	TT	TC	CC	Significance <i>p</i> =
Initial VA (mean logMAR (95% CI))	0.85 (0.68 to 1.02)	0.89 (0.73 to 1.05)	0.83 (0.67 to 0.99)	0.85
Post-treatment VA at 6 months (mean logMAR (95% CI))	0.71 (0.57 to 0.84)	0.73 (0.63 to 0.84)	0.62 (0.50 to 0.74)	0.38
Post-treatment VA at 9 months (mean logMAR (95% CI))	0.75 (0.60 to 0.90)	0.78 (0.67 to 0.88)	0.71 (0.57 to 0.84)	0.70
Number of injections in 9 months (mean (95% CI))	3.3 (2.89 to 3.78)	3.8 (3.54 to 4.16)	3.9 (3.51 to 4.36)	0.09

TABLE 3Recurrent event analysis of interval between injections by *CFHY402H* variant genotypes.

	TC	CC
Hazard Ratio (compared to TT genotype)	1.25	1.37
95% Confidence Interval	0.94 to 1.67	1.01 to 1.87
Significance ($p =$)	0.12	0.04

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