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CLINICAL INVESTIGATION

Complement factor H R1210C among Japanese patients with age-related macular degeneration

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Abstract
Purpose To evaluate the genotype distribution of a rare age-related macular degeneration (AMD)-susceptibility variant, complement factor H (*CFH*) R1210C, among a large Japanese cohort with AMD.
Methods One thousand three hundred and sixty-four Japanese patients with neovascular AMD were evaluated. We screened for *CFH* R1210C (rs121913059) by genotyping with the Taqman method; the mutation was confirmed by Sanger sequencing. We also searched for this mutation in the human genome variant database, which contains the whole-exome sequencing data for 1208 Japanese individuals. The detailed characteristics of patients with this mutation were reviewed.
Results The mean age of the patients was 74.5 years (standard deviation 8.7); men accounted for 71.8 % of the patients. The *CFH* R1210C variant was found in only 1 of the 1364 AMD patients, and was heterozygous (minor allele frequency (MAF) = 0.037 %); it was not found in any of the 1208 individuals in the control group (MAF = 0 %). The patient with *CFH* R1210C was a 70-year-old woman whose main complaint was visual loss in the right eye. Dilated fundus examination, optical coherence tomography, and fluorescein and indocyanine angiography revealed polypoidal choroidal neovasculopathy (PCV), but no drusen in either eye. Despite treatment, her visual acuity had decreased to 1/50 by 6.8 years after her first visit.
Conclusions The *CFH* R1210C variant was found to be rare among Japanese patients with AMD. The patient with the mutation did have the PCV subtype, but no drusen formation. Considering their ethnicity-specific nature, such rare variants should be studied by use of next-generation sequencing for each ethnicity.

Keywords Age-related macular degeneration · Complement factor H · Genetics · Rare variant · R1210C

Introduction

Age-related macular degeneration (AMD) is a major cause of severe, irreversible visual impairment among elderly individuals in developed countries [1–3]. Despite recent advances in anti-VEGF therapy [4, 5], AMD is often difficult to control, owing to deterioration of macular function [6]. The origins of AMD are both environmental, for example smoking [7], and genetic [8–11]. Thus, current smoking increases the odds of AMD development 3.5-fold, and having the TT allele at age-related maculopathy susceptibility 2 (*ARMS2*) A69S (rs10490924) or the CC allele at complement factor H (*CFH*) Y402H (rs1061170) increases it 6.2-fold or 4.1-fold, respectively [12]. Recently, the AMD Gene Consortium performed a genomewide association study (GWAS) and reported 19 AMD-susceptibility single-nucleotide polymorphisms (SNP) [13]. The association of many of these loci with development of AMD among Asian populations has been confirmed, and it is also recognized that their effect can differ between Asian and Caucasian individuals [13, 14].

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Although GWAS achieved great success in identifying common (usually defined as minor allele frequency (MAF) of ≥1 %) AMD-susceptibility SNP, these SNP do not sufficiently explain the heritability of AMD (at best, they explain 65 % of the heritability) [13, 14]. To explain the missing heritability, researchers changed their focus to rare variants. In contrast with common SNP, rare susceptibility variants tend to have larger effects, because they sometimes result in loss of function. Studies using next-generation sequencing have revealed that rare variants located within *CFH*, *C3*, *CFI*, and *C9* confer a high risk of AMD [15–19]; in particular, *CFH* R1210C has a large effect (odds ratio 18.8), and both relatively early-onset and drusen-rich AMD have been reported for individuals with this mutation [19]. This mutation is also known to be responsible for atypical hemolytic uremic syndrome (aHUS) [20] and primary glomerulonephritis [21]. However, its association with AMD has been reported for Caucasian cohorts only, not for other ethnicities. Because rare variants are sometimes over-represented in particular ethnic groups, information about whether this mutation occurs among Japanese populations and causes AMD is of interest, especially considering its large effect. Therefore, in this study, we evaluated 1364 Japanese patients with AMD for the distribution of *CFH* R1210C.

Patients and methods

All procedures adhered to the tenets of the Declaration of Helsinki. The institutional review boards and ethics committees of Kyoto University Graduate School of Medicine, Fukushima Medical University, and Kobe City General Hospital approved the protocols. All patients were fully informed of the purpose and procedures of the study, and written consent was obtained from each patient.

All of the patients were recruited from Kyoto University Hospital, Fukushima Medical University, and Kobe City General Hospital. Comprehensive ophthalmic examinations were conducted, including dilated fundus examination, optical coherence tomography (OCT), fluorescein angiography (FA), and indocyanine angiography (ICGA). Exudative AMD was diagnosed by retina specialists in accordance with the International Classification System for age-related maculopathy [22], as described elsewhere [23]. Patients without a clear diagnosis of AMD subtypes (owing to an older lesion) were excluded from the analysis.

One thousand three hundred and sixty-four patients were included in the study. Genomic DNA samples were prepared from peripheral blood by use of a DNA extraction kit (QuickGene-610L; Fujifilm, Tokyo, Japan). The genotype of *CFH* R1210C (rs121913059) was determined for all samples by use of a custom-made Taqman genotyping assay (Taqman SNP assay with the ABI Prism 7700 system; Applied Biosystems, Foster City, CA, USA). The primer and probe sequences used for this assay were: 5′-AGGTGGACAG CCAAACAGAAG-3′ (forward primer); 5′-AGTTTCCCA TCCCAACATGTTGT-3′ (reverse primer); TGTGTGA GAACGTGATGAA (Taqman probe—VIC labeled); and TGTGTGAGAACATGATGAA (Taqman probe—FAM labeled). This mutation was validated by conventional Sanger sequencing, which was performed with an Applied Biosystems 3130*xl* Genetic Analyzer (Life Technologies, Grand Island, NY, USA). We used the same primer set as in our previous report: Fwd-5′-CCCTAATTCTCATACATTAAA-CATCG-3′ and Rev-5′-GACACAACCGTTAGTTT TCCAG-3′ [19]. To determine the frequency of the mutation among healthy individuals, we referred to the human genetic variation database (HGVD; http://www.genome.med.kyoto-u.ac.jp/SnpDB/; in the public domain). The HGVD contains genetic variations determined by whole-exome sequencing of 1208 Japanese controls.

We reviewed the medical records of individuals with the *CFH* R1210C variant to obtain details of both their clinical course and their disease characteristics. Glomerular filtration rate (GFR) was estimated from serum creatinine levels by using the CKD-EPI equation for Japanese females: $194 \times \mathrm{SCr}^{-1.094} \times \mathrm{Age}^{-0.287} \times 0.739$.

Samples from 1300 of the AMD patients were also genotyped by use of the HumanOmniExpress or HumanExome BeadChip array (Illumina, San Diego, CA, USA) then imputation by use of a 1000-Genomes Project cosmopolitan reference (details are described in the Supplementary Note). Thus, we looked up the genotypes of other AMD-susceptibility SNP whose associations with AMD were confirmed for both Caucasian [13] and Asian [14] populations. These SNP include *ARMS2* (rs10490924), *CFH* (rs800292), *C2/CFB* (rs429608), *C3* (rs2241394), *APOE* (rs4420638), *CETP* (rs3764261), *VEGFA* (rs943080), *TNFRSF10A* (rs13278062), *CFI* (rs4698775), *TGFBR1* (rs334353), and *ADAMTS9* (rs6795735). We constructed a multilocus genetic risk score (GRS) by summing up the number of risk alleles for each SNP, weighted by its reported effect size (log odds ratio). Because rs13278062 in *TNFRSF10A* could not be well imputed, we excluded rs13278062 when constructing the score. The effect sizes used in this analysis are summarized in Supplementary Tables 1 and 2.

Results

Details of the patients' ages, sex, and AMD subtypes are summarized in Table 1

The Taqman assay revealed that only 1 of the 1364 patients with neovascular AMD had the *CFH* R1210C mutation

Table 1 Patients' ($N = 1364$) characteristics

Characteristic	Number
Age, years (mean ± SD)	74.5 ± 8.7
Sex (male:female)	979 (71.8 %):385 (28.2 %)
AMD subtypes	
Typical AMD	578 (42.3 %)
Polypoidal choroidal vasculopathy	721 (52.9 %)
Retinal angiomatous proliferation	65 (4.8 %)

AMD age-related macular degeneration

(MAF = 0.037 %), in contrast with 0.83 % reported for Caucasian patients with AMD [19]. Direct sequencing around this mutation (Fig. 1), performed for this patient, confirmed a heterozygous allele change of CGT to TGT (arginine to cysteine). This mutation was not detected in the 1208 Japanese controls (MAF = 0 %).

The distribution of genetic risk scores for 1300 of the AMD patients is shown in Fig. 2. The patient with the *CFH* R1210C variant had a GRS of 3.54 located at the 47.2 percentile, meaning that the patient had almost median genetic susceptibility to AMD, except for the presence of the *CFH* R1210C mutation.

Case study: patient with AMD and the CFH R1210C mutation

The patient was a 70-year-old Japanese woman who visited Fukushima Medical University Hospital with progressively blurred vision associated with metamorphopsia in the right eye. She had neither a significant medical history nor a history of previous ocular disease. Her family medical history was unavailable. On examination, the best-corrected visual acuity was 18/20 OD and 20/20 OS. No particular abnormality was found in the anterior segment, but moderate cataracts were found in both eyes. Dilated funduscopic examination of the left eye was unremarkable. In the right eye, a hemorrhagic pigment epithelial detachment (PED) with extravagant exudation was present, but drusen were absent (Fig. 3a). FA revealed hyperfluorescence pooling in the PED, and the ICGA clearly revealed a polypoidal lesion at the edge of the PED. Except for high blood sugar (205 mg/dL), the results of the hematologic tests were within normal limits, including an estimated glomerular filtration rate of 74.1 mL/min.

One month after her first visit, the patient's visual acuity had decreased to 8/20 OD because of subretinal hemorrhage; we therefore conducted photodynamic therapy (PDT). After 4 rounds of PDT at intervals of 3–6 months, disease activity was controlled. However, subretinal

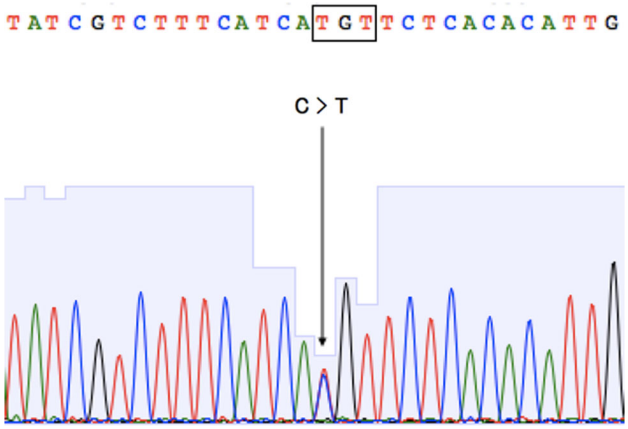

Fig. 1 Sanger sequencing confirmation of *CFH* R1210C. A heterozygous allele change of CGT to TGT (arginine to cysteine) was observed in only 1 of the 1364 Japanese patients with AMD

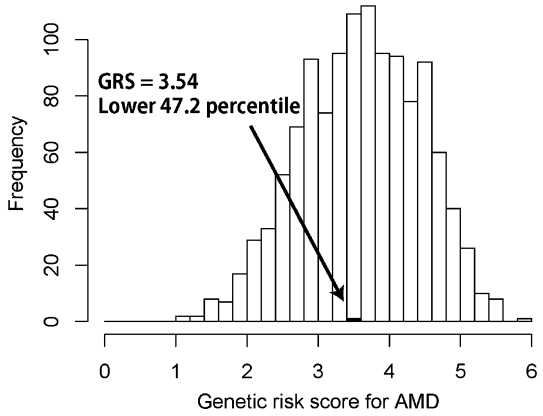

Fig. 2 Distribution of the genetic risk score for Japanese patients with AMD. The genetic risk score were normally distributed. The patient with *CFH* R1210C had a genetic risk score of 3.54, which was in the lower 47.2 percentile of the Japanese patients with neovascular AMD

hemorrhage occurred again 16 months after the final PDT, which caused further visual acuity loss to 1/20 OD. The patient was treated once with intravitreal bevacizumab and twice by focal photocoagulation, which stabilized disease activity but resulted in no visual acuity improvement (visual acuity = 1/50 OD). At her final visit (6 years and 9 months after her initial visit), dilated funduscopic examination revealed several drusen in the left eye. She also had diffuse RPE damage and a massive fibrotic scar in the right eye (Fig. 3b).

Discussion

Age-related macular degeneration is a complex disease the origins of which are both genetic and environmental. Environmental risk factors have been identified in several

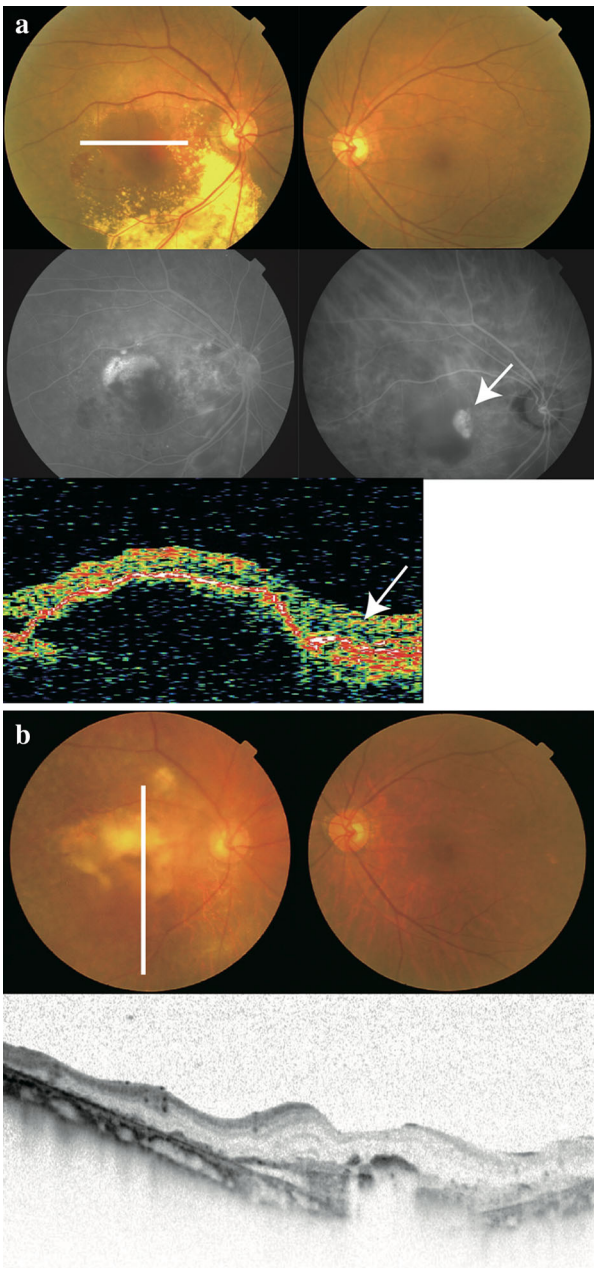

Fig. 3 Case presentation of the patient with AMD and the *CFH* R1210C variant. **a** A 70-year-old Japanese woman visited Fukushima Medical University Hospital with progressively blurred vision associated with metamorphopsia in the right eye. Dilated funduscopic examination of the left eye was unremarkable. In the right eye, there was a hemorrhagic pigment epithelial detachment with extravagant exudation, but no drusen. Fluorescein angiography revealed the classic pattern of choroidal neovascularization, and indocyanine green angiography clearly revealed a polypoidal lesion (arrow). **b** Although 1 treatment using intravitreal bevacizumab and 2 treatments using focal photocoagulation stabilized the disease activity, her visual acuity remained 1/50 OD. At her final visit (6 years and 9 months after the first visit), dilated funduscopic examination revealed several drusen in the left eye and diffuse RPE damage and a massive fibrotic scar in the right eye

epidemiologic studies, and genetic aspects of AMD have also been intensively investigated. In particular, GWAS have contributed substantially to understanding of the effect of common variants [8, 13, 14]. The effect of rare variants has not been elucidated until recently, however. In this study we evaluated one such variant, *CFH* R1210C, and found that it rarely occurs in Japanese patients with neovascular AMD.

CFH R1210C is a missense mutation whose association with AMD was reported in 2011 [19]. Because of an arginine-to-cysteine amino acid change, the C-terminal function of CFH is disrupted, leading to defective binding to C3d, C3b, heparin, and endothelial cells [24, 25]. Although this mutation is also responsible for aHUS and primary glomerulonephritis [20, 21], the only patient who had *CFH* R1210C in our study did not have a medical history of aHUS or renal dysfunction. This is not surprising, given that the penetration of this mutation is not high. A previous study of patients with this mutation found that they, also, did not have these conditions [19]. However, the fact that AMD shares genetic determinants with other diseases and that this mutation was rarely found among a Japanese population, compared with findings for Caucasian populations, is of interest. For example, *CETP* D442G (rs2303790), which increases the risk of AMD and reduces the risk of myocardial infarction, is exclusive to East Asian populations [14, 26]. These observations suggest two things to us. First, rare variants are sometimes ethnicity-specific. Thus, there might be additional rare AMD-susceptible variants within *CFH* that can be found only in Asian individuals. Identifying such variants would facilitate our understanding of the effect of *CFH* in AMD development. Second, rare variants can be associated with several diseases because many of these variants directly affect function. In that respect, rare aHUS susceptibility variants within *CFH* [27] might be good candidates for examination of novel variants for susceptibility to AMD.

Individuals with the *CFH* R1210C variant develop signs of AMD earlier than those without it, with a median age of onset of 65 years (range 35–75 years). In addition, the retinal phenotype for these individuals typically includes numerous small, medium, and large drusen [19]. The patient with *CFH* R1210C identified in this study did not have drusen at the time of diagnosis, however; drusen were not observed until her final visit. Furthermore, her AMD subtype was PCV, which has been predominantly observed among Asian populations. This finding indicates that dysfunction of *CFH* does not solely regulate the retinal phenotype. Retinal phenotypes, for example drusen and polypoidal lesion formation, might arise as a result of an interactive effect between *CFH* and other genes. Although target resequencing, whole-exome sequencing, and whole-

Table 2 Genotype distribution of *CFH* R1210C

AMD subtype	*n*	*CFH* R1210 homozygous	*CFH* R1210C heterozygous	MAF (%)
Typical AMD	578	578	0	0
Polypoidal choroidal vasculopathy	721	720	1	0.069
Retinal angiomatous proliferation	65	65	0	0
Total	1364	1363	1	0.037

AMD age-related macular degeneration, *MAF* minor allele frequency

genome sequencing using next-generation sequencers have been performed for more than 2000 Caucasian individuals with AMD, and have identified several rare AMD-susceptibility variants within known AMD-susceptibility genes [15–19], such sequencing has not been performed for Asian populations. Considering the ethnicity-specific nature of rare variants, high-throughput sequencing should also be applied to Asian patients with AMD; this, with the previous research on Caucasian patients, will help to clarify the mechanisms of AMD and its variable phenotypes (Table 2).

As far as we are aware, this is the first study to evaluate the *CFH* R1210C variant among a large cohort of Japanese patients with AMD. We showed that the *CFH* R1210C variant was present in 1 of the 1364 Japanese patients with AMD. This patient had PCV without initial drusen, which is not a typical phenotype for this variant. The ethnicity-specific nature of rare variants encourages us to perform more analysis with next-generation sequencing among Japanese cohorts with AMD; this may help further elucidate genetic differences between Japanese and Caucasian populations with AMD.

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