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Genotype-phenotype correlation of age-related macular degeneration : influence
of complement factor H polymorphism

THESE

préparée sous la direction du Professeur associé Francis Munier
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RAPPORT DE SYNTHÈSE

Corrélation génotypique-phénotypique de la dégénérescence maculaire liée à l'âge : influence du polymorphisme du gène complement factor H

La dégénérescence maculaire liée à l'âge (DMLA) est une maladie très fréquente qui représente la cause principale de cécité légale chez les sujets de plus de 50 ans en Occident. Bien que l'étiologie exacte de cette affection ne soit pas complètement connue, des facteurs environnementaux et génétiques influencent sa survenue et son évolution. A ce sujet, de récentes recherches ont notamment montré une association marquée à une variante du gène CFH, Y402H. Nous ignorons toutefois si le polymorphisme Y402H est associé à un phénotype particulier de la maladie. Cette étude a pour but d'établir si cette variante du gène CFH est associée à certaines caractéristiques phénotypiques précoces.

L'étude porte sur quatre cent vingt patients atteints de DMLA qui ont été phénotypés sur la base de photographie du fond d'œil (International Classification and Grading system for age-related macular degeneration) et génotypés à partir d'ADN leucocytaire à la recherche de la variante Y402H du gène CFH. Ces données ont ensuite fait l'objet d'une analyse statistique de l'association génotype-phénotype (le génotype de 50 sujets-contrôle a été utilisé pour confirmer l'association du polymorphisme avec la DMLA).

Les résultats obtenus, corrigés pour l'âge et le sexe, montrent un odds ratio (OR) de développer une DMLA de 2.95 en présence d'au moins un allèle à risque C et de 9.05 pour les homozygotes CC. Par contre, aucune influence n'est observée sur les stades de la maladie (précoce-tardif). Une association significative entre le génotype CC et la présence de druses périphériques ($p=0.028$), ainsi que la localisation centrale des druses ($p=0,049$) a été mise en évidence. Aucune autre tendance n'a été dégagée concernant les critères restants (taille, surface totale recouverte, localisation nasale des druses) ou les changements pigmentaires.

Cette étude a permis de confirmer l'association entre la variante Y402H et la DMLA dans la population suisse et de conclure que la variante Y402H du gène CFH présente une association géno-phénotypique pour certaines caractéristiques des druses. Il est probable que d'autres facteurs génétiques encore influencent le phénotype de la DMLA.

De nouvelles recherches seront nécessaires pour préciser l'influence de ces autres facteurs génétiques ou environnementaux, en vue d'une meilleure compréhension de la pathogenèse de cette affection, et pour développer des mesures thérapeutiques et prophylactiques adaptées.

Genotype–phenotype correlation of age-related macular degeneration: influence of complement factor H polymorphism

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ABSTRACT

Background/aims: Complement factor H (CFH) Y402H polymorphism shows a strong association with age-related macular degeneration (AMD). Although the phenotypic concordance of AMD has been shown in sibling/twin studies, little is known about the genotype–phenotype association. In this study, we investigated whether CFH Y402H is associated with early phenotypic features.

Methods: Statistical analysis was performed on 420 patients with AMD with complete clinical and genetic data (graded colour fundus photographs, according to the International Classification and Grading System for AMD and successful testing for CFH Y402H).

Results: In this Swiss population, an OR of 2.95 was confirmed for AMD in the presence of at least one risk C allele and OR of 9.05 for the CC homozygotes, corrected for age and sex. No difference was found between the AMD stages. Patients homozygous for the risk allele showed significant association with peripheral drusen ($p = 0.028$) and for central drusen location ($p = 0.049$). No trend was found for other drusen criteria (size, total surface, location nasal to disc) and for pigmentary changes.

Conclusions: The CFH Y402H polymorphism showed a genotype–phenotype association for some drusen features. Additional genetic factors are likely to influence drusen phenotype.

Age-related macular degeneration (AMD) is the leading cause of severe visual impairment in the Western world among people over 50 years of age due to vision-threatening late complications such as geographic atrophy (GA) and choroidal neovascularisation (CNV).^{1–5}

Numerous genetic^{6,7} as well as environmental factors^{8,9} have been shown to be involved in the pathogenesis of AMD. Family and twin studies, as well as population-based genetic epidemiological studies, have convincingly demonstrated the importance of genetics in AMD,^{10–17} with a heritability estimated to be between 46 and 71%.¹⁷ Phenotypically, impressive concordance of clinical features of AMD (in both its early and late forms) was shown in monozygotic twin studies of AMD,^{17–19} and to a lesser degree in sibling studies.¹⁵

Recently, a number of polymorphisms have been identified for their risk association with AMD.^{6,7} The polymorphism that was first detected is located in the gene for complement factor H (CFH), an important regulator of the complement system. CFH polymorphism Y402H (rs1061170) is one of the two most significant AMD genetic risk

factors described to date,^{20–28} the second being LOC387715 (rs10490924, Ala69Ser).^{24, 29–32}

Investigation of the phenotypic association was concentrated on the late forms of AMD, namely GA and CNV with inconsistent results.^{20, 22, 23, 27, 28, 30, 31, 33–38} However, little is known about the association with early features of AMD.

CFH is also implicated in membranoproliferative glomerulonephritis (MPGN) type II, a renal disorder associated with a particular type of drusen, referred to as “cuticular”. In addition, CFH polymorphism is significantly more common in patients with cuticular drusen, independent of MPGN II.³⁹ Complement factors, including factor H, are known to accumulate within drusen.^{22, 40, 41}

The purpose of the present study was to determine whether or not we could find a genotype–phenotype association in early AMD for the CFH Y402H polymorphism. Such knowledge would increase our understanding of the disease, and should ultimately help to improve its management.

METHODS

Study population: AMD cases

All study participants were recruited from the Jules Gonin Eye Hospital, Lausanne, Switzerland. To be included, patients had to present clear signs of AMD without potentially interfering retinopathy/maculopathy (eg diabetic). They had to be at least 50 years of age at the time the colour photographs were taken; slide or digital cameras were used (Zeiss FK30, Zeiss, Jena, Germany; Canon 60Z/60U, Canon, Utsunomiya, Tochigiken, Japan; and Topcon TRC501A or TRC501X, Topcon, Capelle A/D IJssel, the Netherlands).

The study adhered to the tenets of the Declaration of Helsinki and was approved by the locally appointed ethics committee. All patients gave their informed consent. Initially, 466 unrelated patients were enrolled, of which 41 were ultimately excluded: 24 for incomplete clinical data, 12 because the diagnosis of AMD was not confirmed and five for potentially confounding retinopathy. Therefore, genetic and statistical analysis was performed on 425 patients with AMD (EDTA blood samples were taken for DNA extraction).

Study population: control selection

A control population was selected only for confirmation of the AMD risk association with the described polymorphism, for the Swiss population. Individuals serving as controls were unrelated

Table 1 Study population by allele frequency and genotypic groups

Genotype	Allele frequency		Genotype frequency			Total	p Value	
	T	C	TT	TC	CC		Allele	Genotype
AMD cases*	352 (41.9)	488 (58.1)	71 (16.9)	210 (50)	139 (33.1)	420 (100)	1×10 ⁻⁵	5×10 ⁻⁵
Controls*	65 (65)	35 (35)	19 (38)	27 (54)	4 (8)	50 (100)		
Age of AMD cases (years)†			73.9 (8.8)	76.4 (7.6)	75.4 (7.4)	75.7 (7.8)		0.995
Age of controls (years)†			73.5 (5.4)	75.7 (6.3)	73.8 (7.8)	74.9 (6.2)		

*Values are means (%).

†Values are means (SD).

AMD, age-related macular degeneration.

to the AMD patients, or were spouses. They had to be free of any signs of AMD and other retinopathies/maculopathies. Colour fundus photographs were taken. To reduce the risk of including yet unrevealed AMD patients, only individuals older than 65 years were included. Informed consent was given by all control individuals.

Sixty-three individuals agreed to participate. However, 11 were excluded: seven due to signs of AMD and four who had missing or poor quality photographs (secondary to age-related cataract). Blood samples from the remaining 52 controls were analysed for CFH Y402H polymorphism (EDTA blood samples were taken for DNA extraction).

Fundus grading

Colour fundus photographs were graded before genetic analysis was performed and the results were not communicated to the geneticist. Each eye was graded independently. The reading was done by one of us (I Droz), based on the International Classification and Grading System for AMD.⁴² This classification was chosen rather than the Age-Related Eye Disease Study (AREDS) severity scale^{43,44} or the clinical age-related maculopathy staging (CARMS) system⁴⁵ because our primary interest was the phenotype of AMD rather than progression of the disease in terms of stages. Because of the complexity of the International Classification and Grading System, we considered it necessary to reduce the number of parameters. Drusen criteria were maintained (largest size, predominant size, total surface, distribution), while the classification of pigmentary anomalies was changed to its presence/absence, with foveal or extrafoveal location; GA and CNV were classified as present or absent. The grid template was used as described in the International Classification.⁴²

In terms of AMD stages, we distinguished early/intermediate AMD (extrafoveal GA allowed) from late AMD, classified as central GA or CNV (any sign). As the neovascular form is considered more severe, it was regarded to be dominant over any co-existing GA.

A study by Postel et al,⁴⁶ comparing AMD phenotypes between singleton and multiplex families, found a significant difference between the two groups in terms of the presence of peripheral drusen. We therefore added this parameter of

peripheral drusen (presence/absence), i.e., those located outside of the temporal vascular arcades (>125 µm).

Genotyping

DNA was extracted from peripheral blood using the NucleonBacc2 and the protocol provided by the manufacturer (GE Healthcare Europe, Otelfingen, Switzerland). The single nucleotide polymorphism rs1061170, located in the complement factor H gene and responsible for the Y402H variant, was evaluated after polymerase chain reaction (PCR) amplification and denaturing high-performance liquid chromatography (DHPLC) analysis. PCR was performed with primers forward (5'-TTCITTTTGTGCAAACCTTTGTT-3') and reverse (5'-CCATTGGTAAAACAAGGTGACAT-3'). Thirty-five cycles of PCR were performed (denaturation at 95°C for 1 min; annealing at 58°C for 1 min; extension at 72°C for 1 min). The PCR products were screened by DHPLC (55°C, 57.4% start B, 0.56 time shift and a 1.5 ml/min flow rate).

Statistical analysis

Statistical analyses were done by an epidemiological centre (Center for Clinical Epidemiology, University of Lausanne, Switzerland).

Before analysing the genotype-phenotype association, we set out to replicate the association of CFH Y402H polymorphism with AMD in our population: the relative risk for AMD was analysed for CC homozygotes, CT heterozygotes and for both groups together (presence of at least one risk C allele), correcting for age and sex (logistic regression), comparing patients and controls.

Investigation of phenotypic features was performed in two steps: first, the relative risk for late AMD stages (GA and CNV) was analysed for CC homozygotes and CT heterozygotes, correcting for age and sex, using the more severely affected eye. The grading criteria for drusen and pigmentary changes were then analysed (chi-square test), using the less affected eye. The decision to use the less affected eye was made because grading data were often more complete in the absence of obscuring lesions such as GA or CNV. Concordance of drusen criteria between the two eyes was actually good, with kappa ranging between 0.52 and 0.78.

Table 2 Stage of AMD by allele frequency and genotypic group

Genotype	Allele frequency		Genotype frequency			Total	p Value*	
	T	C	TT	TC	CC		Allele	Genotype
Early/intermediate AMD	135 (43)	177 (57)	26 (16.7)	83 (53.2)	47 (30.1)	156 (100)		
GA (central)	41 (37)	71 (63)	7 (12.5)	27 (48.2)	22 (39.3)	56 (100)	0.26	0.42
CNV	176 (42)	240 (58)	38 (18.3)	100 (48.1)	70 (33.6)	208 (100)	0.86	0.63

Values are n (%).

*p Values comparing late AMD with early AMD (chi-square test).

AMD, age-related macular degeneration; CNV, choroidal neovascularisation; GA, geographic atrophy.

Table 3 Relative risk to develop late stages of age-related macular degeneration (AMD) versus early/intermediate stages, according to the potential risk genotypes compared with the TT homozygotes

Late phenotype	TC			CC		
	OR	95% CI	p Value	OR	95% CI	p Value
Geographic atrophy	1.005	0.37 to 2.66	0.99	1.52	0.55 to 4.15	0.41
Neovascular AMD	0.78	0.42 to 1.41	0.41	0.996	0.53 to 1.88	0.99
Any late AMD	0.82	0.46 to 1.46	0.51	1.07	0.58 to 1.97	0.83

All results are corrected for age and sex.

RESULTS

The average age of AMD patients and of controls was similar (75.7 and 74.9 years, respectively). Among the 425 AMD patients, there were 278 women (65.4%); in the control group, there were 26 women (50%). Genetic results were successfully obtained for all individuals, except for five AMD patients and two controls. Statistical analysis was thus performed on 420 AMD cases and 50 controls.

The three possible genotypes (TT, TC and CC) of the CFH Y402H polymorphism showed the following distribution: of the 420 AMD cases included, 71 (16.9%) had TT, 210 (50%) had TC and 139 (33.1%) had CC (table 1). Of the 50 control subjects, 19 (38%) had TT, 27 (54%) had CT and four (8%) had the CC genotype (table 1).

After correcting for age and sex in a logistic regression analysis, the C allele showed a significant association with AMD in this Swiss population, with an OR of 9.05 (95% CI 2.92 to 27.98; p<0.0001) for CC homozygotes and an OR of 2.00 (95% CI 1.02 to 3.94; p=0.04) for TC heterozygotes compared with TT homozygotes. The OR for the presence of at least one C allele (TC or CC) was 2.95 (95% CI 1.54 to 5.67; p=0.001), with an estimated population attributable risk (PAR) of 55%.

Table 2 shows the distribution of AMD patients according to their disease stage and genotype.

The analysis for the relative risk to develop GA or CNV, according to a given genotype, showed no statistically significant difference for TC heterozygotes and CC homozygotes compared with TT homozygotes (table 3).

Analysis of drusen criteria and pigmentary changes showed the following results (table 4).

No significant difference was found between genetic groups with respect to drusen size (largest and predominant), drusen covered surface, drusen distribution and pigmentary changes. However, peripheral drusen did show a significant association with genotype, being more common in individuals with the risk allele C, particularly if homozygous (p=0.028).

Regrouping of all individuals with circular drusen distribution (relative sparing of the central 500 µm), and comparison with those with central drusen predominance showed a significant trend for central drusen location in patients with the risk allele, particularly if homozygous (p=0.049). Both factors combined remained significantly associated with the risk allele (p=0.030).

DISCUSSION

The CFH Y402H polymorphism (rs1061170) is an important genetic AMD risk factor in the white American population,²⁰⁻²⁸ and this was later confirmed in French,⁴⁷ UK,³⁷ Icelandic,²⁷ Italian³⁸ and Russian⁴⁸ populations. It should be mentioned, however, that the strongest CFH association was found for a different non-coding variant (rs1410996)^{26,30}, the only variant that has also been shown significant in the Japanese population.⁴⁹⁻⁵² In the Chinese population results were inconsistent.^{53,54} The first part of our study confirmed the risk association with

AMD in the Swiss population; the OR was within the reported range (at least one C allele: between 2.45²³ and 4.36;²⁸ C homozygous: between 3.33²³ and 8.35.²⁴) Correspondingly, our calculation of the PAR (55%) is similar to the result of a meta-analysis of the first eight reports (PAR 58.9%).⁵⁵ This confirmation in our population was important before focusing on the analysis of the genotype-phenotype association within the AMD group. Given the agreement with the literature, we considered the relatively small number of controls sufficient for confirming the reported genetic risk association in our population.

Several studies have investigated the influence of CFH Y402H polymorphism on the AMD phenotype in terms of GA or CNV, as a poorly regulated complement cascade due to the CFH polymorphism could well enhance cellular damage, leading ultimately to atrophy or neovascular response.^{41,56,57} In our study, we were not able to confirm any significant difference in the prevalence of either GA or CNV, or early/intermediate AMD, in relation to the Y402H genotype. These results fit well into the inconsistent results reported to date: some investigators have suggested a higher risk for neovascular AMD,^{22,23,35,36} while others found a trend towards atrophic AMD,^{20,28,31,33,34,58} or found like us no difference at all.^{27,30,37,38} It may well be that the influence of Y402H polymorphism on the type of late AMD is minor.

However, there is good evidence for increased risk for progression associated with the CFH polymorphism from two studies with longitudinal data.^{59,60} Similarly, Despret and colleagues found a progressively increasing relative risk for each successive stage of AMD.²⁰ In our study, however, we did not find higher rates of late AMD associated with the CFH Y402H genotype.

In the literature, unspecified genetic influence was shown on early phenotype criteria of AMD, in terms of drusen and pigmentary changes.^{15,17,19} Our investigation on the specific influence of the CFH polymorphism showed a significant association with the presence of drusen outside the vascular arcades and a significant trend for foveal drusen location (as well as the combination of both criteria). These findings might reflect a wider spread distribution of drusen in the CFH risk situation.

Previous reports described extramacular and peripheral drusen in hereditary patterns within families whose older members have advanced AMD,⁶¹ and more common in cases occurring in multiplex families than in patients from singleton families.⁴⁶ However, the only study to look at the relation of drusen characteristics with the genotypes of CFH and LOC387715, found no significant association with peripheral drusen, as well as with all other early phenotypic features of AMD.³⁴

In our study, all other drusen characteristics (largest drusen, predominant drusen, surface covered by drusen, location nasal to the disc) were similarly not associated with the investigated CFH polymorphism. In a sense this is not surprising, as, for a

Table 4 Proportion of individuals with a specific phenotypic feature by genotypic group

	TT	TC	CC	p Value	
				Allele	Genotype
Drusen size (µm)				0.19	0.19
<63	0.050	0.027	0.024		
63–125	0.367	0.225	0.244		
125–250	0.367	0.438	0.366		
>250	0.217	0.310	0.366		
Predominant drusen (µm)				0.97	0.33
<63	0.161	0.096	0.122		
63–125	0.419	0.394	0.431		
125–250	0.274	0.415	0.317		
>250	0.145	0.096	0.130		
Surface covered by drusen (%)				0.12	0.32
<10	0.540	0.465	0.362		
10–25	0.220	0.245	0.362		
25–50	0.180	0.214	0.210		
>50	0.060	0.076	0.067		
Main drusen localisation				0.12	0.33
Central circle	0.480	0.618	0.687		
Intermediate circle	0.300	0.204	0.192	0.025*	0.049*
Outer circle	0.160	0.132	0.101		
Outside the circles	0.060	0.046	0.020		
Peripheral drusen				0.012	0.028
Present	0.300	0.407	0.493		
Absent	0.700	0.593	0.507		
Peripheral drusen and central drusen location				0.017	0.030
Combined	0.086	0.196	0.239		
None or not combined	0.914	0.804	0.761		
Pigment changes				0.64	0.74
Absent	0.482	0.469	0.444		
Extrafoveal	0.107	0.179	0.176		
Foveal	0.411	0.352	0.380		

p Value calculation with chi-square test for the genotype frequency and the allele frequency (data serving for the calculation of the latter not shown).

*p Value for central circle versus intermediate circle, outer circle and outside the circle (CC genotype).

complex genetic disorder such as AMD, other genes may be responsible for early phenotypes of the disease. On the other hand, several observations suggest a role of CFH on drusen phenotype.

First, it was shown that CFH polymorphism increases the risk even for early stages of AMD.^{20,27} Second, drusen were hypothesised to be the result of inflammatory and/or immune-mediated processes,⁶² with accumulation of CFH and various factors of the complement pathway.^{22,40,41} Third, particularly cuticular drusen are strongly associated with CFH polymorphism³⁹ and they are also a typical feature in membranoproliferative glomerulonephritis (MPGN) type II, a disorder in which CFH is implicated. And fourth, the low prevalence and nearly absent impact of the CFH Y402H polymorphism in Asians^{49–51,53,54} coincides with the Asian phenotype of AMD, that is not usually characterised by drusen in its early stages.^{63,64}

We acknowledge that a greater number of patients may be needed to detect the phenotypical influence of genetic polymorphisms. Grading variables of the drusen phenotype may change over time as the disease progresses, thereby confounding phenotype and stage of the disease.

Furthermore, we acknowledge that in our study the absence of data about the smoking status (environmental risk factor for disease susceptibility) precluded statistical correction. However, previous reports found no association with the AMD type,⁶⁵ except for neovascular AMD.⁶⁶

Future research about the genotype–phenotype association in AMD will have to deal with multiple challenges:

- ▶ The potential influence of numerous genes and environmental factors on the phenotype. Careful history-taking, general examination, screening of all known genetic risk factors and a prospective study design are needed.
- ▶ Different stages of the disease may be confounding; longitudinal data may be needed to address this problem.
- ▶ Additional aspects of phenotype could include visual function and age at onset of the AMD. The role of these variables has not yet been established.

Further advances in research about the genotype–phenotype correlation in AMD may prove valuable to better understand of the genetic influence on the pathogenesis of AMD, to develop new prophylactic and therapeutic measures, and ultimately to care for patients in an individualised, and thereby cost-effective way.

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