The spectrum of central serous chorioretinopathy: clinical characteristics, genetic associations and outcome of treatment
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Genetic risk factors in severe, non-severe, and acute phenotypes of central serous chorioretinopathy

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ABSTRACT

Purpose: To study genetic predispositions and differences between severe chronic central serous chorioretinopathy (cCSC), non-severe cCSC, and acute CSC (aCSC).

Methods: 173 severe cCSC patients, 272 non-severe cCSC patients, 135 aCSC patients, and 1385 control individuals were included. Eight single nucleotide polymorphisms (SNPs) were genotyped in the *ARMS2* (rs10490924), *CFH* (rs800292, rs1061170, rs1065489, rs1329428, rs2284664, rs3753394), and *NR3C2* (rs2070951). Additionally, *C4B* gene copy numbers were analyzed.

Results: A significant association in 5 SNPs in the *CFH* gene could be reproduced among severe cCSC patients including rs800292 ($P = 0.0014$, OR = 1.93 [95%CI = 1.51-2.47]), rs1065489 ($P = 2.22 \times 10^{-4}$, OR = 0.49 [95%CI = 0.34-0.72]), rs1329428 ($P = 0.001$, OR = 1.89 [95%CI = 1.49-2.40]), rs2284664 ($P = 1.21 \times 10^{-4}$, OR = 1.65 [95%CI = 1.28-2.13]), and rs3753394 ($P = 6.10 \times 10^{-4}$, OR = 0.61 [95%CI = 0.46-0.81]). Carrying three *C4B* copies was protective for severe cCSC ($P = 0.001$, OR= 0.29 [95%CI = 0.14-0.61]). No significant differences in allele frequencies could be found among the CSC phenotypes.

Conclusions: Acute CSC, non-severe cCSC, and severe cCSC all showed a similar association with the *CFH* and *C4B* genes, and the three phenotypes could not be distinguished based on the genetics. This shows that, despite the differences in clinical presentation and severity, there is an overlap in the genetic predisposition of different CSC phenotypes. Non-genetic factors may play a more important role in determining the clinical course of CSC.

INTRODUCTION

Central serous chorioretinopathy (CSC) is a chorioretinal disease, characterized by serous fluid accumulation in the subretinal space, often affecting the macula with subsequent visual impairment.[1] The underlying pathophysiology of CSC is not fully understood. However, a congested, hyperpermeable, and leaking choroid, together with a damaged and dysfunctional retinal pigment epithelium (RPE) are thought to underlie the subretinal fluid (SRF) accumulation in CSC.[2]

At least two different CSC phenotypes can be distinguished: acute and chronic CSC. Acute CSC (aCSC) is generally considered self-limiting with a near-complete visual recovery, thus not requiring treatment in most cases. In contrast, chronic CSC (cCSC) often has persistent SRF with more extensive atrophic RPE changes, in which treatment can be beneficial.[1] There is no consensus on the duration threshold that distinguishes acute and chronic CSC, but an arbitrary period of four to six months duration of active disease (SRF leakage) is often considered for the definition of chronicity.[1] Apart from chronic SRF leakage, patients with cCSC may present with a wide spectrum of retinal abnormalities. In mild cCSC cases there are limited areas of RPE atrophy, few RPE detachments, and a circumscribed area of leakage.[3] More severe cCSC cases show widespread or multifocal (or both) areas of RPE atrophy, more numerous RPE detachments, diffuse areas of leakage, and intraretinal cystoid degeneration.[4-6] Moreover, this spectrum of severe cCSC was previously shown to have the worst visual prognosis among all cCSC cases, even after treatment and complete resolution of SRF.[7] Therefore, severe cCSC may be considered a distinct clinical subgroup within the spectrum of CSC.

Recently, specific single nucleotide polymorphisms (SNPs) in the *age-related maculopathy susceptibility 2* (*ARMS2*), the *complement factor H* (*CFH*), and the *nuclear receptor subfamily 3 group C member 2* (*NR3C2*) genes were found to be associated with the risk of cCSC.[8-10] Genomic copy number variations in the *complement component 4* (*C4B*) gene were also shown to be associated with cCSC.[11] As aCSC, 'non-severe' cCSC, and 'severe' cCSC appear substantially distinct CSC subgroups with regard to clinical manifestation and prognosis (Figure 1), these different CSC forms may also have different genetic risk profiles.

In the present study, we analyzed the association of SNPs in the *ARMS2*, *CFH*, *NR3C2* genes, and copy numbers of *C4B* gene, in a cohort of cCSC patients who showed a severe disease presentation based on previously published disease characteristics.[7] In addition, we analyzed and compared the association of the aforementioned risk SNPs between three Caucasian CSC subgroups, including aCSC, cCSC without characteristics of severity, and cCSC patients with severity characteristics.

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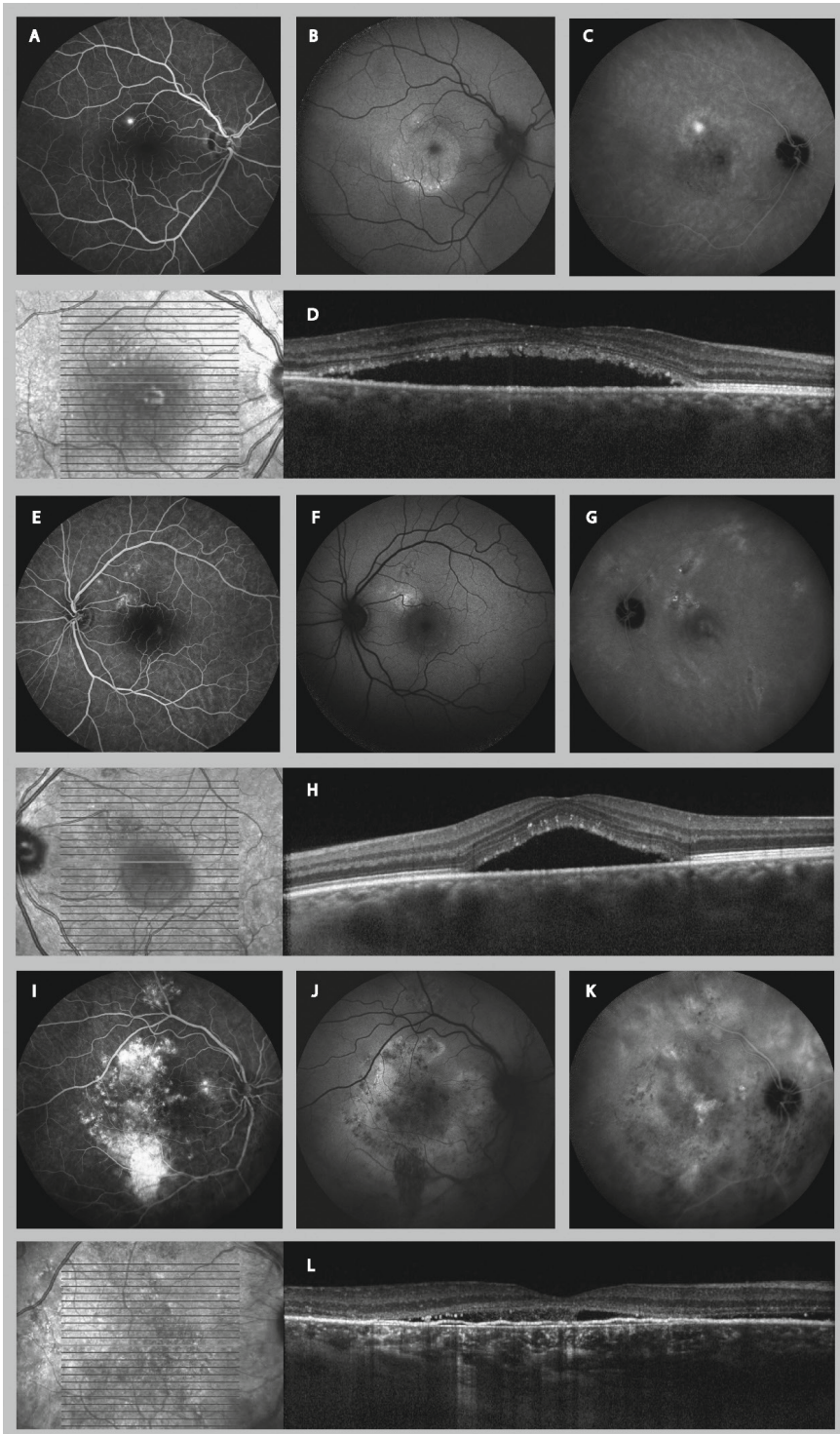
<- Figure 1. Clinical features on multimodal imaging in different central serous chorioretinopathy (CSC) phenotypes. The right eye of a 34-year-old male with acute CSC (aCSC) is shown in A-D. In E-H the left eye of a 43-year-old male patient with non-severe chronic CSC (cCSC) is shown. In I-L the right eye of a 61-year-old male patient with severe cCSC is shown. Fluorescein angiography (FA) imaging revealed a single "hot spot" of leakage and no atrophic retinal pigment epithelium (RPE) changes in the aCSC patient (A). FA in the non-severe cCSC showed a leakage spot and multifocal small areas of RPE changes (E), while in the severe cCSC case large and widespread RPE atrophy and diffuse leaking areas were seen (I). On mid-phase indocyanine green angiography (ICGA) in the aCSC case, a small hyperfluorescent lesion was observed at the site of the "hot spot" on FA (C). In contrast, ICGA in the severe and non-severe cCSC patients showed more extensive multifocal hyperfluorescent changes (G, K). Fundus autofluorescence (FAF) imaging showed a mix of granular hyper-autofluorescent and hypo-autofluorescent changes which were most prominent in the severe cCSC patient (B,F,J). Optical coherence tomography (OCT) scan at first presentation revealed a subretinal serous fluid (SRF) accumulation and subretinal debris in all patients (D, H, L). Furthermore, a typical irregular shallow RPE detachment was present in the severe cCSC case (L), which is often observed in combination with chronic SRF leakage.

3.2

MATERIALS AND METHODS

In total, 173 Caucasian subjects with a severe cCSC phenotype were included, originating from four tertiary referral centers: 65 patients from the Department of Ophthalmology of Leiden University Medical Center (Leiden, the Netherlands), 67 patients from Radboud University Medical Center (Nijmegen, the Netherlands), 24 patients from the Rotterdam Eye Hospital (Rotterdam, the Netherlands), and 17 patients from University Eye Hospital of Cologne (Cologne, Germany).

Patients were phenotyped by two experienced retina specialists (SY, CJFB). For phenotyping, a complete ophthalmological examination was used including fundoscopy, optical coherence tomography (OCT), fluorescein angiography (FA), and when available indocyanine green angiography (ICGA). Caucasian patients were included in the severe group of cCSC when they had a history of active disease for over 6 months, in combination with at least one of the following abnormalities: 1. Cumulative areas of larger than five optic disc diameters (DD) of diffuse atrophic RPE alterations visible on mid-phase FA; 2. At least 2 "hot spots" of leakage on mid-phase FA; 3. An area of diffuse fluorescein leakage larger than one DD on mid-phase FA, without an evident leaking focus; 4. Presence of posterior cystoid retinal degeneration assessed on OCT.[7, 12] Subjects were excluded when there was a suspicion of a (secondary) choroidal neovascularization, aneurysmal choroidal vasculopathy, age-related macular degeneration, multifocal choroiditis, retinal vascular occlusions, or high myopia. The presumably steroid-induced CSC cases (steroid use within 3 months prior to CSC diagnosis) were not excluded from analysis.

The cohort of severe cCSC was genetically compared to a cohort of 272 Caucasian patients with non-severe cCSC, who had a history of persistent disease but did not have any of the 4 previously mentioned characteristics of severity. Additionally, severe cCSC was compared to 135 Caucasian patients with aCSC, defined as a combination of: 1. Documented serous

SRF accumulation on OCT; 2. A single focal leakage point on FA; 3. Atrophic RPE alterations limited to less than one DD in size. The control group included Caucasian individuals enrolled in the European Genetic Database (EUGENDA; www.eugenda.org), in whom no signs of macular disease were found on multimodal imaging, and 176 subjects included in the blood bank of the Radboud University Medical Center. Approval for this study was obtained at the local institutional review boards in all participating centers, and the study adhered to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all subjects prior to blood collection for genetic analysis.

Single nucleotide polymorphism genotyping

DNA was isolated from peripheral blood by using standard procedures. The most relevant genetic variants to be analyzed were chosen based on findings in earlier genetic studies in CSC, and included the following variants: *ARMS2* (rs10490924), *CFH* (rs800292, rs1061170, rs1065489, rs1329428, rs2284664, rs3753394), and *NR3C2* (rs2070951), and copy number variations in the *C4B* gene.[8-11] KASP assays (LGC Genomics; Berlin, Germany) were used for SNP genotyping, as described previously and according to manufacturer's instructions. A 7900HT Fast Real-Time PCR system (Applied Biosystems by Life Technologies, Austin, TX, USA) was used to read out the genotyping data. Data analysis was performed with SDS (version 2.4, Applied Biosystems). A TaqMan genotyping assay (Hs07226350_cn, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) with RNaseP as a reference assay was used to measure C4B gene copy numbers, as described previously.

Statistical analysis

The allele frequency of the SNPs in severe cCSC patients was compared to either unaffected controls, non-severe cCSC, or aCSC using a 2-sided Pearson's Chi-square test (IBM SPSS Statistics, version 22, SPSS Inc., Chicago, IL, USA). The C4B copy numbers distribution was compared with a 2-sided Fisher's exact test. Additionally, a logistic model correcting for gender was designed and two copies of *C4B* were set as a reference.[11] *P*-values <0.0056 were considered statistically significant after a Bonferroni correction for multiple testing for 9 variants. Haplotype analysis correcting for gender was performed to assess the combined effect of the selected six variants in *CFH* using R (R Core Team, v3.0.2) with the haplo.stats package (v1.7.7). As a reference, the two most frequent haplotypes were used in the haplo. glm command to determine odds ratios (ORs) for the haplotypes with a frequency >5%, and the aggregate of the haplotypes with a frequency <5%.

RESULTS

In the present study, we included 173 patients with severe cCSC (mean age: 54 ± 10 years, 151 (87%) males), 272 patients with non-severe cCSC (mean age: 51 ± 10 years, 216 (79%) males), and 135 patients with aCSC (mean age: 47 ± 10 years, 92 (68%) males). The demographic characteristics are summarized in Table 1.

Table 1 Demographic characteristics of the study population and controls per tested gene

	Severe cCSC	Non-severe cCSC	aCSC	Controls ARMS2 & CFH	Controls C4B	Controls NR3C2
Number of subjects	173	272	135	826	250	1385
Number of males	151 (87%)	216 (79%)	92 (68%)	424 (51%)	198 (79%)	635 (46%)
Mean age ± SD (years)	54 ± 10	51 ± 10	47 ± 10	64 ± 12	51 ± 10	51 ± 10

ARMS2 = age-related maculopathy susceptibility 2; *CFH = complement factor H*; *C4B = complement component 4*; *NR3C2 = nuclear receptor subfamily 3 group C member 2*.

Association with SNPs in the ARMS2, NR3C2, and CFH genes

No significant association was found with the rs10490924 variant in *ARMS2* gene, nor with the rs2070951 variant in the *NR3C2* gene in the severe cCSC group after correction for multiple testing (Table 2). Also, no difference was observed in allele frequencies of these tested variants when comparing severe cCSC with non-severe cCSC or aCSC (Table 3). An association could be found in six tested variants in the *CFH* gene in the severe cCSC group(Table 2). Associations of five *CFH* variants remained significant after correction for multiple testing: rs800292 (P = 0.0014, OR = 1.93 [95% Confidence Interval (CI) = 1.51-2.47]), rs1065489 (P = 2.22 × 10^{-4}, OR = 0.49 [95%CI = 0.34-0.72]), rs1329428 (P = 0.001, OR = 1.89 [95%CI = 1.49-2.40]), rs2284664 (P = 1.21× 10^{-4}, OR = 1.65 [95%CI = 1.28-2.13]), rs3753394 (P = 6.10× 10^{-4}, OR = 0.61 [95%CI = 0.46-0.81]). No difference was observed when comparing allele frequencies of the six tested variants in the *CFH* gene between severe cCSC and either non-severe cCSC or aCSC (Table 3).

Association with CFH haplotypes

Five haplotypes in the *CFH* gene with a frequency above 5% and an aggregate of the haplotypes with a frequency lower than 5% were identified. When using the most common haplotype (H1) as a reference and correcting for gender, severe cCSC showed an association with H2, H3, H4, H5, and the low frequency aggregated haplotypes (Table 4). However, only H2 remained significant after correction for multiple testing, which was risk carrying for severe cCSC (P = 0.001, OR = 1.73 [95%CI = 1.24-2.41], Table 4). Using the H2 haplotype as a reference, H1 and H3 were both associated with severe cCSC after correction for multiple testing, caxrrying a protective effect (P = 0.0013, OR = 0.58 [95%CI = 0.41-0.81] and P = 4.14 x 10^{-6}, OR = 0.30 [95%CI = 0.18-0.50], respectively) (Table 4). When comparing the haplotype frequencies of severe cCSC to this frequencies in non-severe cCSC and aCSC, no significant differences were found after correction for multiple testing (Table 5 and Table 6).

Table 2 Analysis of eight single nucleotide polymorphisms in severe chronic central serous chorioretinopathy

Single nucleotide polymorphism (locus)	Alleles in controls (major/ minor)	Severe cCSC (n)	MAF in severe cCSC	Controls (n)	MAF among controls	Unadjusted allelic P	Allelic odds ratio (95% CI)
rs10490924 (ARMS2)	G/T	171	0.187	812	0.217	0.214	0.83 (0.62-1.11)
rs2070951 (NR3C2)	C/G	172	0.494	1385	0.468	0.350	1.11 (0.89-1.39)
rs800292 (CFH)	G/A	172	0.372	798	0.235	0.0014*	1.93 (1.51-2.47)
rs1061170 (CFH)	T/C	172	0.282	803	0.353	0.012	0.72 (0.56-0.93)
rs1065489 (CFH)	G/T	172	0.096	794	0.177	2.22×10^{-4}*	0.49 (0.34-0.72)
rs1329428 (CFH)	C/T	171	0.588	787	0.429	0.0010*	1.89 (1.49-2.40)
rs2284664 (CFH)	C/T	171	0.316	805	0.219	1.21×10^{-4}*	1.65 (1.28-2.13)
rs3753394 (CFH)	C/T	171	0.202	800	0.293	6.10×10^{-4}*	0.61 (0.46-0.81)

* 2-sided $P < 0.00556$ was considered significant after correction for multiple testing.

ARMS2 = age-related maculopathy susceptibility 2; CFH = complement factor H; MAF = minor allele frequency; NR3C2 = nuclear receptor subfamily 3 group C member 2.

Table 3 Comparison of allele frequencies in severe cCSC versus non-severe cCSC, and aCSC

SNPs (locus)	Group 1*		Group 2†		Group 3‡		Group 2 vs 1		Group 3 vs 1	
	Severe cCSC (n)	MAF	Non-severe cCSC (n)	MAF	aCSC (n)	MAF	Unadjusted allelic P	Allelic odds ratio (95% CI)	Unadjusted Allelic P	Allelic odds ratio (95% CI)
rs10490924 (ARMS2)	171	0.187	243	0.193	132	0.174	0.821	0.96 (0.67-1.37)	0.683	1.09 (0.72-1.66)
rs2070951 (NR3C2)	172	0.494	269	0.520	132	0.538	0.447	0.90 (0.69-1.18)	0.216	0.82 (0.59-1.13)
rs800292 (CFH)	172	0.372	245	0.296	133	0.320	0.021	1.41 (1.05-1.89)	0.177	1.26 (0.90-1.77)
rs1061170 (CFH)	172	0.282	245	0.320	133	0.259	0.235	0.83 (0.62-1.13)	0.534	1.12 (0.78-1.61)
rs1065489 (CFH)	172	0.096	244	0.133	134	0.119	0.101	0.69 (0.44-1.08)	0.350	0.78 (0.47-1.31)
rs1329428 (CFH)	171	0.588	244	0.510	133	0.579	0.0275	1.37 (1.04-1.81)	0.828	1.04 (0.75-1.43)
rs2284664 (CFH)	171	0.316	244	0.275	134	0.287	0.199	1.22 (0.90-1.65)	0.448	1.14 (0.81-1.62)
rs3753394 (CFH)	171	0.202	242	0.273	131	0.263	0.0192	0.67 (0.48-0.94)	0.073	0.71 (0.48-1.0)

3.2

* Group 1: severe chronic central serous chorioretinopathy; † Group 2: non-severe chronic central serous chorioretinopathy; ‡ Group 3: acute central serous chorioretinopathy.

2-sided P <0.00556 was considered significant after correction for multiple testing.

ARMS2 = age-related maculopathy susceptibility 2; CFH = complement factor H; MAF = minor allele frequency; NR3C2 = nuclear receptor subfamily 3 group C member 2.

Table 4 *Complement factor H haplotypes in severe cCSC*

Haplotypes	Variants						HF among controls	HF among severe cCSC	Unadjusted allelic P	Allelic odds ratio (95% CI)	Unadjusted allelic P	Allelic odds ratio (95% CI)
	rs3753394	rs800292	rs1061170	rs2284664	rs1329428	rs1065489						
H1	C	G	C	C	C	G	0.329	0.255	Base	Base	**1.25 x 10^{-3}***	0.58 (0.41-0.81)
H2	C	A	T	T	T	G	0.209	0.300	**0.0013***	1.73 (1.24-2.41)	Base	Base
H3	T	G	T	C	C	T	0.158	0.065	0.012	0.52 (0.31-0.87)	**4.14 x 10^{-6}***	0.30 (0.18-0.50)
H4	C	G	T	C	T	G	0.133	0.164	0.030	1.57 (1.05-2.35)	0.615	0.90 (0.61-1.34)
H5	T	G	T	C	T	G	0.072	0.094	0.011	1.91 (1.16-3.15)	0.697	1.10 (0.67-1.82)
Rare	*	*	*	*	*	*	0.098	0.122	0.028	1.65 (1.06-2.57)	0.829	0.95 (0.61-1.49)

* P < 0.0083 was considered significant after correction for multiple testing.

HF = haplotype frequency; MAF = minor allele frequency.

Table 5 *Complement factor H haplotypes in non-severe cCSC versus severe cCSC*

Haplotypes	Variants						HF among non-severe cCSC	HF among severe cCSC	Unadjusted allelic P	Allelic odds ratio (95% CI)	Unadjusted allelic P	Allelic odds ratio (95% CI)
	rs3753394	rs800292	rs1061170	rs2284664	rs1329428	rs1065489						
H1	C	G	C	C	C	G	0.301	0.255	Base	Base	0.170	0.77 (0.53-1.12)
H2	C	A	T	T	T	G	0.262	0.300	0.170	1.29 (0.9-1.87)	Base	Base
H3	T	G	T	C	C	T	0.104	0.065	0.216	0.69 (0.38-1.24)	0.034	0.53 (0.3-0.95)
H4	C	G	T	C	T	G	0.111	0.164	0.038	1.66 (1.03-2.67)	0.302	1.28 (0.8-2.05)
H5	T	G	T	C	T	G	0.115	0.094	0.897	0.97 (0.59-1.6)	0.034	0.75 (0.45-1.23)
Rare	*	*	*	*	*	*	0.108	0.122	0.281	1.32 (0.8-2.17)	0.946	1.02 (0.62-1.68)

$P < 0.0083$ was considered significant after correction for multiple testing.

HF = haplotype frequency; MAF = minor allele frequency.

3.2

151

Table 6 *Complement factor H* haplotypes in aCSC versus severe cCSC

Haplotypes	Variants						HF among aCSC	HF among severe cCSC	Unadjusted allelic P	Allelic odds ratio (95% CI)	Unadjusted allelic P	Allelic odds ratio (95% CI)
	rs3753394	rs800292	rs1061170	rs2284664	rs1329428	rs1065489						
H1	C	G	C	C	C	G	0.249	0.255	0.690	1.10 (0.70-1.72)	Base	Base
H2	C	A	T	T	T	G	0.272	0.300	Base	Base	0.690	0.91 (0.58-1.43)
H3	T	G	T	C	C	T	0.102	0.065	0.110	0.60 (0.32-1.12)	0.068	0.55 (0.29-1.04)
H4	C	G	T	C	T	G	0.164	0.164	0.940	0.98 (0.58-1.65)	0.683	0.89 (0.52-1.53)
H5	T	G	T	C	T	G	0.114	0.094	0.628	1.91 (1.16-3.15)	0.435	0.79 (0.44-1.43)
Rare	*	*	*	*	*	*	0.100	0.122	0.677	1.14 (0.62-2.10)	0.903	1.04 (0.56-1.93)

$P < 0.0083$ was considered significant after correction for multiple testing.

HF = haplotype frequency; MAF = minor allele frequency.

C4B copy number determination

The distribution of *C4B* copy numbers was significantly different in severe cCSC compared to controls after correction for multiple testing (*P* = 0.0020) (Figure 2). A logistic regression model showed that carrying three *C4B* copies was protective for severe cCSC (*P* = 0.001, OR= 0.29 [95%CI = 0.14-0.61]) (Table 7). The distribution of *C4B* copy numbers was not significantly different between severe cCSC, non-severe cCSC, and aCSC groups (Figure 2). In addition, the overall logistic regression model for effect size was not significant when comparing severe cCSC with non-severe cCSC (*P* = 0.665), or when comparing severe cCSC with aCSC (*P* = 0.551) (Table 8 and Table 9).

Table 7 Logistic regression model for *C4B* load in severe cCSC patients

Overall significance model P = 0.007				
	Controls (n = 250)	**Severe cCSC (n = 164)**	**P**	**Odds ratio (95% CI)**
Male sex	**198 (79%)**	**143 (87%)**	**0.010**	**0.48 (0.27-0.84)**
C4B copy number				
0	6 (2.4%)	4 (2%)	0.781	0.83 (0.23-3.04)
1	55 (22%)	51 (31%)	0.225	1.33 (0.84-2.12)
2	142 (57%)	99 (60%)	Base	Base
3	44 (18%)	10 (6%)	**0.001***	0.29 (0.14-0.61)
4	3 (1.2%)	0	0.999	NA

* *P* <0.0055 was considered significant after correction for multiple testing.
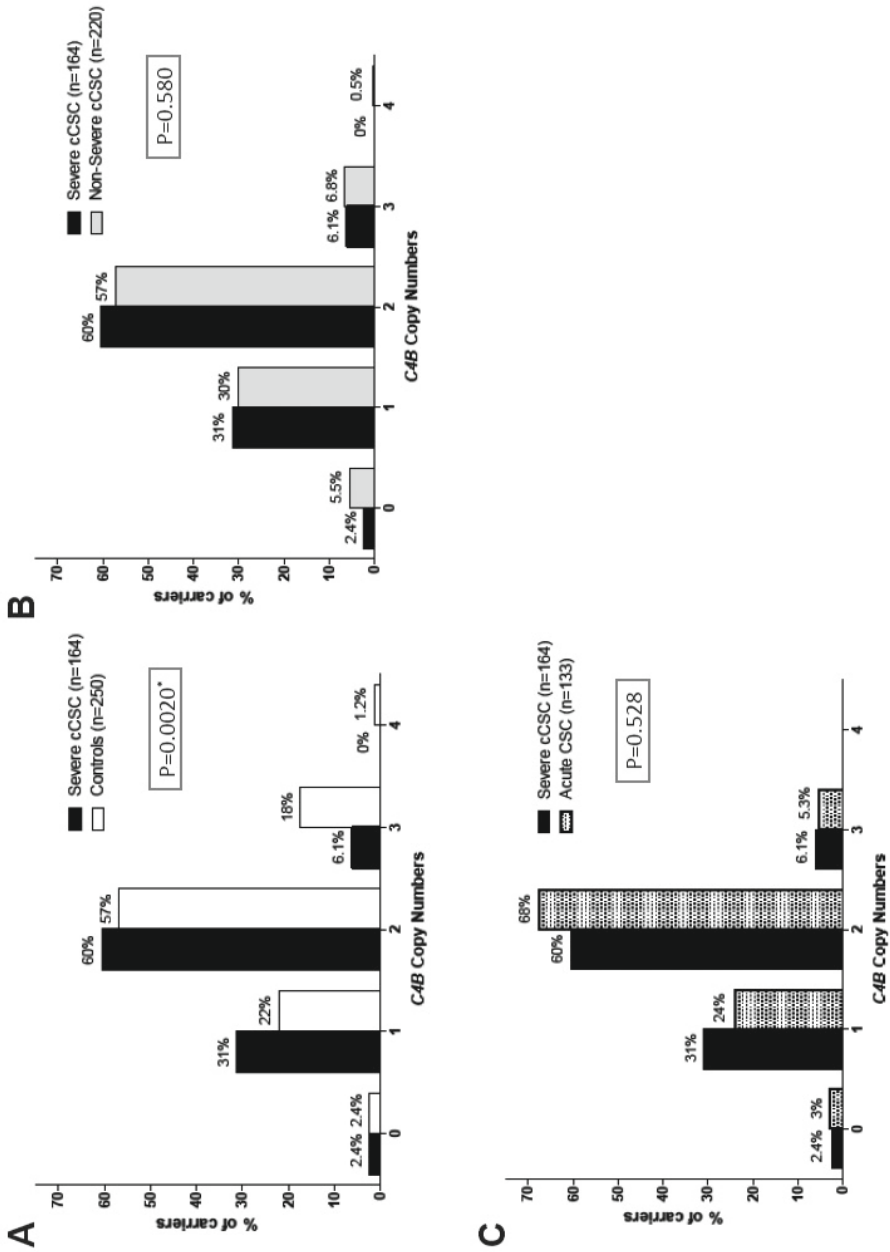cCSC = chronic central serous chorioretinopathy; CI = confidence interval; *C4B = complement component 4*; NA = not annotated.

Table 8 Logistic regression model for *C4B* load in severe versus non-severe cCSC

Overall significance model P = 0.665				
	Non-severe cCSC (n = 220)	**Severe cCSC (n = 164)**	**P**	**Odds ratio (95% CI)**
Male sex	**216 (79%)**	**143 (87%)**	**0.043**	**0.56 (0.32-0.98)**
C4B copy number				
0	12 (5.4%)	4 (2%)	0.131	0.41 (0.13-1.31)
1	66 (30%)	51 (31%)	0.878	0.97 (0.61-1.52)
2	126 (57%)	99 (60%)	Base	Base
3	15 (6.8%)	10 (6%)	0.678	0.83 (0.36-1.95)
4	1 (0.4%)	0	1	NA

P <0.0055 was considered significant after correction for multiple testing.
C4B = complement component 4; NA = not annotated.

Figure 2. Distribution of *C4B* copy numbers among severe chronic central serous chorioretinopathy (cCSC), non-severe cCSC, acute CSC (aCSC), and controls.

Table 9 Logistic regression model for *C4*B load in severe cCSC versus aCSC

Overall significance model P = 0.551				
	aCSC (n = 133)	Severe cCSC (n = 164)	P	Odds ratio (95% CI)
Male sex	**91 (68%)**	**143 (87%)**	**1.26 x 10^{-4}**	**0.32 (0.17-0.57)**
C4B **copy number**				
0	4 (3.0%)	4 (2%)	0.622	0.70 (0.17-2.90)
1	32 (24%)	51 (31%)	0.194	1.43 (0.83-2.46)
2	90 (68%)	99 (60%)	Base	Base
3	7 (5.2%)	10 (6%)	0.794	1.15 (0.41-3.20)

P < 0.0055 was considered significant after correction for multiple testing.
C4B = complement component 4; NA = not annotated.

DISCUSSION

There is a wide variety in clinical presentation of CSC, ranging from aCSC to severe chronic CSC,[1, 7, 12-14] and it is unclear whether these subgroups are different with regard to pathogenesis and genetic background. In the present study, we analyzed specific genetic risk factors in severe cCSC patients and compared them to non-severe cCSC and aCSC patients. Our data showed that in patients with severe cCSC, three variants (rs800292, rs1329428, and rs2284664) in the *CFH* gene were significantly associated with an increased risk of the disease, while two variants (rs1065489 and rs3753394) were protective. Also, having three copies of the *C4B* gene was protective against severe cCSC. However, no differences were identified between severe, non-severe and acute CSC phenotypes.

A comparison of the genetic associations in the three phenotypic subgroups indicated similar risk and protective profiles in the *CFH* gene variants, *CFH* haplotypes, and *C4B* gene copy numbers. Interestingly, although the groups were not significantly different, the genetic effect size, in terms of protective or risk-conferring odds ratios, was systematically larger in the severe cCSC subgroup compared to non-severe cCSC and aCSC. This was also true when comparing the genetic effect size of *CFH* variants rs800292, rs1329428, and rs1065489 in severe cCSC with cCSC patients in literature.[9] Severe cCSC may therefore have a stronger genetic predisposition than milder CSC subtypes. Our findings indicate that there is a significant overlap in the known genetic risk factors and therefore likely also pathophysiological overlap between CSC subtypes, despite clinical differences.

A role for the complement system, and the *CFH* gene in particular, in the pathogenesis of CSC was suggested previously based on genetic association studies.[8, 9, 11] Our present study confirms this association in all three CSC phenotypic subtypes. As the choroid and choriocapillaris play a central role in the pathogenesis of CSC, while complement activity

is abundant in choroidal tissue,[15] complement system dysregulation may be a key factor in CSC disease mechanism. A range of variants in genes involved in the complement system have also been identified in age-related macular degeneration.[16, 17] In contrast to age-related macular degeneration, no systemic complement abnormalities were found in a relatively small group of cCSC patients.[18, 19] Local complement system effects may be more important in CSC, rather than systemic complement system abnormalities. However, larger studies on systemic complement differences in cCSC patients are necessary.

CSC patients share certain clinical characteristics with age-related macular degeneration, such as macular fluid leakage and RPE abnormalities, as well as possible complication of choroidal neovascularization,[20] but there are also clear differences such as an earlier age at onset, an absence of drusen, the presence of pachychoroid, and association with steroid use. The *CFH* variants reported in this study appear to have opposite effects in CSC compared to age-related macular degeneration, which may point to a different role of the complement system in the pathophysiology of these diseases as suggested before.[8] In our current cohorts, we could not replicate the associations with the *ARMS2* gene and *NR3C2* gene variants as demonstrated previously.[8, 10] This lack of a significant association may be explained by the smaller sample size of the subgroups.

In the present study, a possible role of other, currently unknown, genetic variants cannot be excluded. Other factors may have a more prominent role than genetic factors in determining the course and severity of the disease. Daruich et al. suggested that older age (>40 years), presence of high (>50 µm) RPE detachments, and a thickened (>500 µm) choroid are significantly correlated with a prolonged episode of aCSC.[21] Long-term steroid use has been suggested not only to increase the risk of CSC but also to cause a more severe bilateral chronic disease with multiple RPE leaking sites, more extended areas of RPE atrophy, and even bullous retinal detachments.[22-24] Piccolino et al. have shown that presence of posterior cystoid retinal degeneration, which was considered a sign of severity in our study, is specifically associated with steroid use, longer duration of symptoms and subretinal fibrin accumulation.[12] Furthermore, severe cCSC presentations were previously described in pregnant women,[25] and among certain ethnic groups.[26] Our findings suggest that the profile of known genetic risk SNPs between phenotypically different CSC patients is similar, and therefore it is likely that other factors such as described above determine disease course and outcome.

In conclusion, associations between *CFH* genetic variants and *C4B* copy numbers and severe CSC were demonstrated, but no marked genetic differences were found between acute, non-severe, and severe chronic phenotypes of CSC in the tested variants. This study indicates that different phenotypes of CSC may not develop due to genetic predisposition, at least among the currently known CSC-associated *CFH* variants. Presumably, other non-genetic risk factors such as environmental factors, or currently unknown genetic variants may play a role in the clinical course of CSC. Future genetic and clinical studies in larger cohorts

may provide important clues about the different risk factors associated with CSC disease severity.

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3.2

Conflict of interest

The authors report no financial/conflicting interests in this work.

REFERENCES

1.   Daruich A, Matet A, Dirani A, et al. Central serous chorioretinopathy: Recent findings and new physiopathology hypothesis. *Prog Retin Eye Res* 2015;48:82-118.

2.   Yannuzzi LA, Slakter JS, Sorenson JA, et al. Digital indocyanine green videoangiography and choroidal neovascularization. *Retina* 1992;12(3):191-223.

3.   Breukink MB, Dingemans AJ, den Hollander AI, et al. Chronic central serous chorioretinopathy: long-term follow-up and vision-related quality of life. *Clin Ophthalmol* 2017;11:39-46.

4.   Iida T, Yannuzzi LA, Spaide RF, et al. Cystoid macular degeneration in chronic central serous chorioretinopathy. *Retina* 2003;23(1):1-7; quiz 137-8.

5.   Castro-Correia J, Coutinho MF, Rosas V, et al. Long-term follow-up of central serous retinopathy in 150 patients. *Doc Ophthalmol* 1992;81(4):379-86.

6.   Yannuzzi LA, Shakin JL, Fisher YL, et al. Peripheral retinal detachments and retinal pigment epithelial atrophic tracts secondary to central serous pigment epitheliopathy. *Retina* 1984;91 (12):1554-72.

7.   Mohabati D, van Rijssen TJ, van Dijk EHC, et al. Clinical characteristics and long-term visual outcome of severe phenotypes of chronic central serous chorioretinopathy. *Clin Ophthalmol* 2018;12:1061-1070.

8.   de Jong EK, Breukink MB, Schellevis RL, et al. Chronic central serous chorioretinopathy is associated with genetic variants implicated in age-related macular degeneration. *Ophthalmology* 2015;122(3):562-70.

9.   Miki A, Kondo N, Yanagisawa S, et al. Common variants in the complement factor H gene confer genetic susceptibility to central serous chorioretinopathy. *Ophthalmology* 2014;121(5):1067-72.

10.   van Dijk EHC, Schellevis RL, van Bergen M, et al. Association of a haplotype in the NR3C2 gene, encoding the mineralocorticoid receptor, with chronic central serous chorioretinopathy. *JAMA Ophthalmol* 2017;135(5):446-51.

11.   Breukink MB, Schellevis RL, Boon CJ, et al. Genomic copy number variations of the complement component C4B gene are associated with chronic central serous chorioretinopathy. *Invest Ophthalmol Vis Sci* 2015;56(9):5608-13.

12.   Piccolino FC, De La Longrais RR, Manea M, et al. Posterior cystoid retinal degeneration in central serous chorioretinopathy. *Retina* 2008;28(7):1008-12.

13.   Otsuka S, Ohba N, Nakao K. A long-term follow-up study of severe variant of central serous chorioretinopathy. *Retina* 2002;22(1):25-32.

14.   von Winning CH, Oosterhuis JA, Renger-van Dijk AH, et al. Diffuse retinal pigment epitheliopathy. *Ophthalmologica* 1982;185(1):7-14.

15.   Whitmore SS, Sohn EH, Chirco KR, et al. Complement activation and choriocapillaris loss in early AMD: implications for pathophysiology and therapy. *Prog Retin Eye Res* 2015;45:1-29.

16.   Boon CJ, van de Kar NC, Klevering BJ, et al. The spectrum of phenotypes caused by variants in the CFH gene. *Mol Immunol* 2009;46(8-9):1573-94.

17.   Geerlings MJ, de Jong EK, den Hollander AI. The complement system in age-related macular degeneration: A review of rare genetic variants and implications for personalized treatment. *Mol Immunol* 2017;84:65-76.

18. van Dijk EHC, Tsonaka R, Klar-Mohamad N, et al. Systemic complement activation in central serous chorioretinopathy. *PLoS One* 2017;12(7):e0180312.

19. Kersten E, Paun CC, Schellevis RL, et al. Systemic and ocular fluid compounds as potential biomarkers in age-related macular degeneration. *Surv Ophthalmol* 2018; 63(1):9-39.

20. Peiretti E, Ferrara DC, Caminiti G, et al. Choroidal neovascularization in Caucasian patients with longstanding central serous chorioretinopathy. *Retina* 2015;35(7):1360-7.

21. Daruich A, Matet A, Marchionno L, et al. Acute central serous chorioretinopathy: factors influencing episode duration. *Retina* 2017;37(10):1905-15.

22. Gass JD, Little H. Bilateral bullous exudative retinal detachment complicating idiopathic central serous chorioretinopathy during systemic corticosteroid therapy. *Ophthalmology* 1995;102(5):737-47.

23. Quillen DA, Gass DM, Brod RD, et al. Central serous chorioretinopathy in women. *Ophthalmology* 1996;103(1):72-9.

24. Stefaniotou M, Vourda E, Katsanos A, et al. Multifocal central serous chorioretinopathy associated with steroids in a patient with myasthenia gravis. *Case Rep Ophthalmol* 2013;4(2):1-6.

25. Maggio E, Polito A, Freno MC, et al. Multimodal imaging findings in a case of severe Central Serous Chorioretinopathy in an uncomplicated pregnancy. *BMC Ophthalmol* 2015;15:183.

26. Desai UR, Alhalel AA, Campen TJ, et al. Central serous chorioretinopathy in African Americans. *J Natl Med Assoc* 2003;95(7):553-9.

3.2